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The Status of the Genus Taxus In Kentucky

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THE STATUS OF THE GENUS *TAXUS* IN KENTUCKY

By

Robert Reed Pace

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Date 4-7-2015

The Status of the Genus *Taxus* in Kentucky

By

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Bachelor of Science

Eastern Kentucky University

Richmond, Kentucky

2009

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Eastern Kentucky University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 2015

DEDICATION

This thesis is dedicated to my wife,
Elli Pace, who shared my journey in
pursuing both undergraduate and
graduate degrees at Eastern Kentucky University.

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Last, but by no means least, I would like to acknowledge Elli Pace, my wife, for her assistance, patience, and support during the writing of this thesis.

ABSTRACT

This study involved a detailed study of the genus *Taxus* in Kentucky. A thorough examination was conducted, including a review of the literature, examination of field and herbarium specimens from both native and non-native species, microscopic analysis of leaf ultrastructure, chemical analysis of taxane content, and the construction of GIS models to predict the occurrence of the native species. In the review and examination of morphological features, it was found that the best features for separation of the taxa were plant height, the number of the rows of stomata per abaxial leaf band, and the location of papillose cells on the leaf epidermis. In particular, the SEM studies showed that stomatal bands are a reliable way of differentiating native and non-native *Taxus* species within Kentucky. A key to the taxa was prepared, as well as descriptions of the species. The chemical analysis failed to uncover any reliable differences between taxa utilizing only five taxanes. GIS models were prepared for 13 counties in eastern Kentucky, and these predicted the most likely occurrence of *Taxus canadensis* in each portion of the county. This study documented three species of *Taxus* that occur in Kentucky, *T. baccata*, *T. canadensis*, and *T. cuspidata*. *Taxus canadensis* is the only native species, considered to be a glacial relict, and is currently listed as a state threatened species. The other two species occur only rarely in nature as escapes from cultivation, likely from the spread of seeds by birds. There is no evidence of hybridization between native and non-native species. It was concluded that microhabitat requirements for *T. canadensis* are very restrictive, and that ongoing climate change may impact Kentucky's native population of *T. canadensis*.

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CHAPTER I

INTRODUCTION

The genus *Taxus* (yew) is represented by 1 native species in Kentucky, 3 native species in the United States, and 24 species worldwide. About 55 varieties have been described. The species are notoriously difficult to distinguish by the morphological characters and geographic differences that have traditionally been used to separate the taxa. The species are all so similar that they have been, at times, considered to belong to a single variable species (Pilger 1903). In recent years micromorphological characters, epidermal cell structure in particular, have been used to compare populations. The yews are also well known for their toxic foliage and as a source of metabolically active compounds useful in the treatment of certain cancers.

The three traditionally recognized taxa in the United States include one rare species endemic to the Appalachicola area of Florida (*Taxus floridana* Nuttall ex Chapman), a widespread species of northeastern United States and southeastern Canada that becomes rare near its southern limits in Kentucky and Tennessee (*T. canadensis* Marshall), and a species of far western United States (*T. brevifolia* Nuttall). There is also a native species of yew in Central America, *T. globosa* Schlectendahl.

Many Asian and European species of yew, as well as hybrids, are cultivated in the United States, and these species may occasionally occur spontaneously in habitats away from cultivated plants. Examples of cultivated yews include *Taxus*× *media* Rehder, *Taxus cuspidata* Seibold and Zuccarini, *Taxus* × *hunnewelliana* Rehder, and *Taxus baccata* L.

This study was initiated to find answers to several questions related to the genus *Taxus* in Kentucky:

1) What is the distribution and habitat and taxonomic status of the native *Taxus canadensis* in Kentucky?

2) Are non-native species of *Taxus* escaping to Kentucky woodlands?

3) How similar in micromorphological features are native and non-native species of *Taxus* in Kentucky, and can these features be useful in distinguishing taxa?

4) How similar in chemical profiles are native and non-native species of *Taxus* in Kentucky, and can these features be useful in distinguishing taxa?

5) Can useful keys be prepared, based on macromorphological and micromorphological features, to separate the native and non-native taxa in Kentucky?

6) Can habitat modeling be prepared to assist in locating populations of *Taxus canadensis* and to understand the unusual distribution of this species within Kentucky?

CHAPTER II

LITERATURE REVIEW

According to Hageneder et al. (2001), the family Taxaceae consists of six extant genera: *Amentotaxus*, *Austrotaxus*, *Cephalotaxus*, *Pseudotaxus*, *Taxus*, and *Torreya*, and the following information is based on this reference. *Amentotaxus* is a subtropical genus commonly called the catkin yews and are native to Southeast Asia. The genus *Austrotaxus* are found only in New Caledonia and consists of a single species. The genus *Cephalotaxus* are commonly known as the Plum Yews, and houses eleven species that are endemic to China. The genus *Pseudotaxus* consists of a single species, commonly called the White Berry Yew and is endemic to Southern China. The genus *Torreya* is common called the Nutmeg Yews and contains six species. Four species of the genus *Torreya* are endemic to Asia, with two being found in North America. *Torreya* and *Taxus* are the only two genera of Taxaceae native to North America.

The genus *Taxus* was first described in 1753 by Linnaeus, with his description of the European species *Taxus baccata* (Linnaeus 1753). Linnaeus considered the Canadian populations as conspecific with *T. baccata*, but Marshall (1785) separated these North American populations as *Taxus canadensis*. Nuttall described *Taxus brevifolia* in his North American Sylva (Nuttall 1842–1849), and he also described *T. floridana* (in Chapman 1860). The other species of *Taxus* currently recognized were all described in the eighteenth and nineteenth centuries. Opinions on the numbers of species in the genus have varied widely, from one (Linnaeus 1753, Pilger 1903) to 24 (Spjut 2007). In addition, many varieties have been described, including *Taxus baccata* ‘fastigiata’, *Taxus* *baccata* ‘repandens’, *Taxus cuspidata* ‘nana’, etc. (Cope 1998). Two hybrid species, *Taxus* × *media* and *Taxus* × *hunnewelliana* are also known (Cochran 2014). These hybrids have been extensively bred for the horticultural trade yielding innumerable varieties, such as *Taxus* × *media* ‘brownii’ (Cope 1998).

Genetic studies of *Taxus* have become popular as more scientists are looking at the genes involved in Taxol production (Onrubia et al. 2011) and their regulation, along with exploration of kinship (Chybicki et al. 2011). Phylogenetic studies of *Taxus* (Hao 2008, Robertson 1907) haven’t been overly productive due to a lack of consistent nomenclature being applied to the species along with disparity between circumscriptions for the various species. Collins et al. (2003) examined the relationship between the varieties of hybrids (*Taxus* × *media* and *Taxus* × *hunnewelliana*) and their parent species. This study confirmed the parentage documented historically from the Hunnewell estate and Hicks Nursery in the early 1900s (Cochrane 2014, Hatfield 1922). Li et al. (2001) examined the phylogenetic relationship between most species of *Taxus* but didn’t include hybrids. Allison (1991, 2008) looked at sex expression of *T. canadensis*, which is the only monecious *Taxus* in North America. Allison (1990) reveals that germination rates of *T. canadensis* tends to drop when pollen is most dense. An exact rationale for this behavior hasn’t been concluded. Allison (1993) reports that self-fertilization within yews is often quite common, with males cones being more prevalent on younger branches, and female cones on branches two years old. Allison (1992) also reports that deer have major impact on reproductive success of *T. canadensis* via herbivory. By reducing the populations of *T. canadensis*, it forces the plant to rely more on self-fertilization (Allison 1993). Deer may prefer the more tender young shoots of *T. canadensis* (Holmes et al.

2009, Conover & Kania 1988), which houses the mostly male pollen cones, thus reducing the amount of pollen.

The major problem in understanding *Taxus* classification has to do with a general lack of uniformity in the circumscription of *Taxus* species. Morphology and physiography continue to dominate the taxonomic treatments of *Taxus* (Cope 1998, Spjut 2007). The scant use of phylogenetic analysis at the species level has left many questions regarding relationships/kinship between *Taxus* species questionable or unanswered.

The species of *Taxus* are usually grouped into sectional categories (Spjut 2007), with the North American species being placed in two different groups. *Taxus globosa*, *Taxus floridana*, and *Taxus brevifolia* belong to the *Wallichiana* group and subgroup, and *Taxus canadensis* being placed in the *Baccata* group. The non-native species of *Taxus* commonly cultivated in KY, *Taxus cuspidata*, and *Taxus baccata*, are placed in the *Chinensis* subgroup of the *Wallichiana* group and *Baccata* group respectively.

Various evidences have been used to elucidate the taxa. Geography played a significant role in separation of the taxa into species. This method was preferred by Linnaeus (1753) and Cope (1998). Morphological, geographical, and chemical studies of *Taxus* have been employed more recently by Richard Spjut (2007) to elucidate the taxa. Spjut's approach of comparing both geography, chemistry, and microstructures of the leaves provided a rational for the biogeography of the genus, except in regards to the hybrids which are mostly ignored. Phylogenetic examination of *T. canadensis*, *T. baccata*, *T. cuspidata*, along with the hybrids (*T.* × *media* and *T.* × *hunnewelliana*) examined by Collins et al. (2003) gave insight into the phylogenetics and origin of the hybrid species. Another phylogenetic examination by Li et al. (2001) provided relationship/kinship between most *Taxus* species. Unfortunately the data from Li et al. (2001) was not conclusive in discerning relationships between North American species and those of Europe/Asia. Li et al. (2001) hypothesized that multiple separations and reintroductions between species over time may be responsible for the confounding phylogenetic analysis.

Fossil evidence suggests that family Taxaceae originated from a primitive group of conifers called "Taxads" around 250 MYA during the early Triassic period (Hageneder et al. 2007). The earliest fossil of a *Taxus* species, *Taxus rediviva*, was dated to around 200 MYA, and found across the land mass of that period (Hageneder et al. 2007). Continental drift eventually isolated *Taxus* to the holoarctic regions during the late Cenezoic era (~64 MYA) (Hageneder et al. 2007). *Taxus baccata* first appearance in the fossil record dates to around 16 MYA (Hageneder et al. 2007).

Yew species have a long history of associations with humans (Burrows and Tyrl 2001). Many superstitions surround the plants, going back to ancient times in Egypt, Greece, Rome, and Europe. The branches were a symbol of mourning, and the wood was used in funeral pyres. The branches were among the best for constructing bows and arrows. It was used in sacred rites by the Druids, and later was often planted in cemeteries and churchyards. The species are very long-lived, and some specimens in English churchyards are known to be over 1000 years old (Sterry 2007). Adding to its mystery was its reputation for being toxic, especially to horses and cattle, but also to humans. It has been called the most dangerous shrub both in Europe and in America. Both fresh and dried foliage and bark are extremely toxic, and can be fatal within a few hours of ingestion. Livestock readily eat the evergreen leaves, especially in the winter.

Most human cases occur in children who are attracted to the bright red seeds (there seems to be some dispute on their toxicity) or when deliberately eaten by adults in suicide attempts.

The species have also been used for a variety of herbal remedies and similar uses (Moerman 1998). *Taxus canadensis* was used by Native Americans of the northeastern United States as a drug (antirheumatic, poultice, abortifacient, gastrointestinal problems, gynecological aid, and others), a food (a fermented beverage from the seeds and leaves) and for making a green dye from the leaves (Moerman 1998).

Taxus has been the subject of many chemical studies, mainly because the genus is the source of the chemotherapeutic drug Paclitaxel (Taxol®) (Windels and Flaspohler 2011, Banerjee et al. 2008, Cameron & Smith 2008, Cody et al. 2005, Senger et al. 2006, Shi & Kiyota 2005, Shi et al. 2003, 2004, Walker et al. 1994, Wang et al. 2010, 2011, Watcheung et al. 2011, Zhang et al. 2008). Paclitaxel is a valuable drug used in the treatment of breast, lung, and ovarian cancers. Paclitaxel is produced via three methods; direct extraction from *Taxus* (Windels and Flaspohler 2011); via chemical modification of the precursor 10-deacetylbaccatin (Walker et al. 1994); and via plant cell fermentation's production of 10-deacetylbaccatin, which is then chemically modified to form Paclitaxel. The direct extraction method requires the destructive removal of bark from the plant. This yields a crude chemical cocktail that will be later refined to produce pure paclitaxel. The semisynthetic pathway involves the chemical modification of the paclitaxel precursor compound 10-deacetylbaccatin, resulting in pure paclitaxel (Walker et al. 1994). The semisynthetic pathway is non-destructive, as only the needles of the plant can be harvested. Paclitaxel, and 10-deacetylbaccatin, are found to vary in concentration amongst the different species of *Taxus*. The highest concentration of Paclitaxel and 10-deacetylbaccatin are found in the *T. brevifolia* (Hageneder et al. 2007), and these populations are currently in decline throughout their Pacific coastal distribution because of the over-harvesting that occurred prior to the discovery of the semisynthetic synthesis for paclitaxel. The paclitaxel precursor 10-deacetylbaccatin can be extracted from most species of *Taxus*; including *T. canadensis* (Windels and Flaspohler 2011, Wang et al. 2011, Watcheung et al. 2011). The plant cell fermentation method of producing 10-deaccetylbaccatin doesn't require the continual harvesting of *Taxus* plant material, and as such offers protection for existing *Taxus* populations.

Pharmaceutical research into Taxol and other taxanes often is directed toward finding species or cultivars of *Taxus* that have the highest taxanes or Taxol content (Shi et al. 2003, 2004), or to discover novel taxanes (Senger et al. 2006; Shi and Kiyota 2005) or secondary metabolites (Saxena and Jain 2009). In addition to discovering new taxanes present with *Taxus*, there is also interest in the synthesis of either existing or novel taxanes (Walker et al. 1994).

The quantification of taxanes within *Taxus* has historically been achieved via the use of High Pressure Liquid Chromatography (HPLC) coupled with mass spectrometry (MS) (Cameron and Smith 2008). This technique allows the separation of the taxanes from the other compounds present within the plant material along with measurement of relative taxanes content. A newer technique (described by Cody et al. 2005) in the measurement of relative taxanes content utilizes Direct Analysis in Real Time Mass Spectrometry (DART MS). This technique has the advantages of little to no sample preparation with real time results. DART MS has been used to identify and quantify the concentration of taxanes within *Taxus wallichiana* (Banerjee et al. 2008).

T. canadensis differs from other *Taxus* species by a predominance of asexual reproduction. This yew is also the only member of the Taxaceae that is almost exclusively monecious, bearing both male and female cones in the leaf axils during summer (Windels and Flaspohler 2011). The reproductive structure of all *Taxus* is a small cone (~4 mm) enveloped in a red aril, each containing a single seed. The aril and cone are the only part of *Taxus* that are considered non-toxic (Windels and Flaspohler 2011). Seeds are dispersed predominantly by ingestion of the seeds by birds and mammals and subsequent deposition in their feces, but also just by gravity (Windels and Flaspohler 2011). *Taxus* seeds show low germination, which coupled with slow growth and sensitivity to environmental disturbance, make the genus susceptible to local extirpation (Hageneder et al. 2007). *T. canadensis* within Kentucky are found mostly in small populations, formed either by clonal colonies via the rooting of their prostrate limbs or by seeds being locally distributed by gravity. *T. baccata* has restrictions on seed germination near the parent plant due to an unknown inhibitory means (Devaney et al. 2014). It is unknown whether *T. canadensis* have similar regenerative restrictions.

Young and Young (1992) provided additional information on the seeds and seed germination of *Taxus* species. They noted that most species produced good set crops every year, some every few years. Seeds can be stored for several years at prechilling temperatures in a moist medium. They are noted that for some species that seeds germinate better if passed through the digestive system of birds. Laboratory germination involves the use of warm stratification and prechilling, and that there is some evidence that the natural germination inhibitors can be leached by culture in a liquid nutritive medium.

Taxus species are a highly utilized in horticulture (Cope 1998). The variety of growth patterns and ability to be pruned and utilized as topiaries have made them popular for landscapers (Cope 1998, Welch 1979). As such nurseries have historically had issues with growing *Taxus* from seed due to chemical inhibition and low seed germination rates (Zarek 2007, Melzack and Watts 1982). Another issue with cultivation of *Taxus* revolves around their sluggish growth (Hageneder et al. 2007). One solution to these problems with *Taxus* cultivation has been the extensive use of vegetative propagation in cultivation (Hageneder et al. 2007). An advantage of vegetative propagation, in which a clipping of the plant is used to produce a clone of the parent plant, is that one avoids germination issues entirely. A disadvantage of using vegetative propagation is that the vegetatively propagated specimens do not exhibit the same growth patterns as that of the parent stock (Hageneder et al. 2007). Plants vegetatively propagated do not develop a dominant leader stem, instead will develop a more multi-stemmed and sprawling growth pattern (Hageneder et al. 2007). These morphological differences between the vegetative derived plants and their parental stock can confound identification (Spjut 2007). This makes growth pattern a useless trait in diagnosing vegetatively propagated specimens. To further complicate cultivation, germination of seedlings are inhibited via an unknown means by the parent (Devaney et al. 2014). As such seedlings do not sprout from cones near the parent plant. Hybrid species such as the plethora of cultivars currently used in the horticultural trade, e.g. *Taxus* × *media*, have been discovered to have impaired meiosis, reducing the viability of their sexual reproduction (Collins et al. 2003).

Available taxonomic manuals and atlases and websites provide information on *Taxus* species in Kentucky and the United States, and some address the question of whether or not non-native species have become naturalized in the country. The most significant world treatment of *Taxus* is by Spjut (2007), Overview of the Genus *Taxus* (Taxaceae): The Species, Their Classification, and Female Reproductive Morphology, at http://www.worldbotanical.com/TAXNA.HTM. Spjut (2007) also provides a treatment with keys for North America at http://www.worldbotanical.com/Key%20NA%20Species.htm. The major taxonomic resource on *Taxus* in North America is the treatment in Flora North America (Hils 1993). This reference provides detailed description of the genus, pertinent literature citations, keys to the genera and species, species descriptions, and notes on their seed maturation period, their habitats, and their distributions. It is noted in this reference that "detailed study of the genus (not neglecting the cultivated representatives) is much needed and long overdue." There is also a comment that extralimital (non-native) species of *Taxus* are not known to naturalize in North America, but that spontaneous saplings of exotic species may occur within the range of *Taxus canadensis*, probably being spread by bird droppings. *Taxus canadensis* is the only species of the genus listed as occurring naturally in Kentucky.

Other references specific to Kentucky are those by Wharton and Barbour (1973), Medley (1993), Jones (2005), Clark and Weckman (2008), and Campbell and Medley (2014). Wharton and Barbour (1973) described *Taxus canadensis* as occurring in "three colonies, each in a different, moist gorge near the western edge of the Pottsville Escarpment … a northern relict... persisted since Pleistocene glaciation when it received refuge in valleys and coves south of the ice sheet." Medley (1993) listed seven counties for *T. canadensis* in Kentucky (Carter, Jackson, Lee, Menifee, Pulaski, Rowan, and Wolfe), and noted literature records from the Edmonson/Barren county area, based on Hussey (1876). Medley (1993) also noted that *T. cuspidata* was observed as a rare escape on a limestone bluff above the Ohio River floodplain in Jefferson County. Jones (2005) also listed only *T. canadensis* for the state, but commented that "introduced species such as *T. cuspidata*, the Japanese yew, and *T. baccata*, English yew, are frequently planted in KY and may occasionally escape to disturbed woodlands." Clark and Weckman (2008) mapped *T. baccata* as an "extremely rare escape in mixed woods" in Whitley County, and mapped *T. canadensis* as occurring in seven counties in eastern Kentucky (Carter, Jackson, Menifee, Powell, Pulaski, Rowan, and Wolfe). The Clark and Weckman distribution map differs from Medley in the listing of Powell County instead of Lee County. Campbell and Medley (2014) mapped the same counties as Medley, and also indicated literature reports from Edmonson and Owsley Counties. The Kentucky State Nature Preserves (2014) currently lists Carter, Jackson, Lee, Menifee, Pulaski, Rowan, and Wolfe counties for *Taxus canadensis*.

One potential threat for *Taxus canadensis* is the escape of non-native *Taxus* into the wild that may introduce intra-generic competition via hybrids. Two species of non-native *Taxus* have been observed to escape into the wild within Kentucky: *Taxus baccata* and *Taxus cuspidata* (Medley 1993, Campbell and Medley 2014). These two plants are seldom confused with *T. canadensis* due to size differences in adult specimens, but within saplings a taxonomic identification would require microscopic examination. Thus far these escaped non-native *Taxus* have been observed as single specimens and not viable populations (Medley 1993, Campbell and Medley 2014). This reduces any potential threat of these non-native species escaping. Birds are most likely the means behind these non-native escapes. Escape outside of Kentucky have been documented in Pennsylvania (Rhoads et al. 2000), New York (Glenn 2013), Massachusetts (Cullina et al. 2011), and New Jersey (Glenn 2013). Of note is that the most common cultivated *Taxus* within Kentucky is *Taxus* × *media*, but it has yet to be documented as escaping into the wild.

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CHAPTER III

MATERIALS AND METHODS

Field and Herbarium Studies for Morphological and Chemical Analyses

The objective of field studies and herbarium studies was to locate populations of *Taxus* for sampling of foliage and seeds to be used in chemical and microscopic analyses. A list of known occurrences of *Taxus* species in Kentucky was provided from the databases of the Kentucky State Nature Preserves Commission (KSNPC 2015). A collecting permit was obtained from the United States Department of Agriculture Forestry Service to collect *T. canadensis* within Daniel Boone National Forest. Most plant material utilized in this research was obtained from specimen loans from regional herbaria (Table 1). These specimens are individually cited in Appendix A. For live plant specimens (Table 2), samples were obtained by taking one clipping from the plant, usually under 10 cm in length. For collection of the colonial specimens, up to 10 cm samples were obtained from the larger more robust plants along the periphery and center of the plant colony. A single GPS coordinate is recorded for each isolated individual plant, and multiple GPS coordinates are recorded at the vertices of the colonies periphery, along with the center of the colony (Delorme Earthmate PN-40, Yarmouth ME). Plants found in the field were visually inspected in the field for the presence of reproductive structures and fruit. If plants contain reproductive structures or fruit, then these were included in the samples taken. Samples along with a 3d barcode were inserted into a zip closed plastic bag. These fresh specimens were placed in a refrigerator as soon after collection as possible. These samples (Table 2) were processed via chemical analysis within 72 hours after collection.

The objective of herbarium work was to obtain specimens from regional herbaria to determine if any additional specimen locations could be uncovered (other than those documented in previous studies) and to provide specimens for macroscopic and microscopic comparisons. Specimens were borrowed from Northern Kentucky University (KNK), totaling 30 specimens, the University of Tennessee (TENN), totaling 15 specimens, the University of Kentucky (KY), totaling 15 specimens, and West Virginia University (WVA), totaling 94 specimens. These specimens, together with 18 specimens from Eastern Kentucky University (EKY), provided the basis for herbarium studies. Upon arrival, specimen loans were frozen at -40 °C for no less than three days. After freezing the specimens were stored in an insect proof cabinet, and all sheets were photographed with a 2d barcode temporarily affixed to the specimen sheet. Dried plant material was sampled for scanning electron microscopy by removal of two leaves from the specimen sheet, preferably from any paper packet attached to the sheet. The specimen sheet's barcode was then inserted into a sample vial containing the two leaves.

Table 1. Table of taxa examined during microscopic analysis derived from various regional herbaria.

Taxon	**# of specimens**
Taxus baccata Linnaeus	11
Taxus brevifolia Nuttall	22
Taxus canadensis Marshall	112
Taxus cuspidata Siebert & Zuccarini	12
Taxus floridana Nuttall ex Chapman	7
Taxus globosa Nuttal ex Chapman	1
Taxus mairei (Lemée & Leviellé) Shiu-Ying Hu ex Liu	1
Taxus × *hunnewelliana* Rehder	1
Taxus × *media* Rehder	4
Total:	**171**

Table 2. Samples collected for chemical and morphological analysis.

Taxon	State	County	Locality	Collector	Collection #
Taxus baccata L.	Kentucky	Madison	Backyard of 203 Moberly Ave	Robert Pace	SOTINKY1
Taxus × *media* Rehder	Kentucky	Madison	East side of 3rd street near intersection of Woodland Ave.	Robert Pace	SOTINKY2
Taxus × *media* Rehder	Kentucky	Madison	East side of 3rd street between Main st. and Irvine Road	Robert Pace	SOTINKY3
Taxus × *media* Rehder	Kentucky	Madison	Northeast corner of Jones building on EKU's campus	Robert Pace	SOTINKY4
Taxus × *media* Rehder	Kentucky	Madison	In between Coates and Jones building on EKU's campus	Robert Pace	SOTINKY5
Taxus × *media* Rehder	Kentucky	Madison	In between Coates and Jones building on EKU's campus	Robert Pace	SOTINKY6
Taxus × *media* Rehder	Kentucky	Madison	Near main library on EKU's campus	Robert Pace	SOTINKY7
Taxus × *media* Rehder	Kentucky	Madison	Corner of Moore building on EKU's campus	Robert Pace	SOTINKY8
Taxus canadensis Marsh.	Kentucky	Wolfe	Along Middle Fork Red River	Robert Pace	SOTINKY26
Taxus canadensis Marsh.	Kentucky	Wolfe	Along Middle Fork Red River	Robert Pace	SOTINKY27
Taxus canadensis Marsh.	Kentucky	Wolfe	Along Middle Fork Red River	Robert Pace	SOTINKY28
Taxus canadensis Marsh.	Kentucky	Wolfe	Along Middle Fork Red River	Robert Pace	SOTINKY29
Taxus × *media* Rehder	Kentucky	Powell	Slightly North of parking area on Cottage Rd.	Robert Pace	SOTINKY33
Taxus × *media* Rehder	Kentucky	Powell	South side of hemlock lodge	Robert Pace	SOTINKY34
Taxus × *media* Rehder	Kentucky	Powell	North side of hemlock lodge	Robert Pace	SOTINKY35
Taxus cuspidata Sieb. & Zucc.	Kentucky	Fayette	Backyard of 1727 Courtney Ave, Lexington KY	Robert Pace	SOTINKY36
Taxus × *media* Rehder	Kentucky	Menifee	Cliff 0.6mi NW from bridge over Middle Fork of Red River	Robert Pace	SOTINKY37
Taxus × *media* Rehder	Kentucky	Madison	Hedge to the left of the front of Roark building on EKU's campus	Robert Pace	SOTINKY41
Taxus × *media* Rehder	Kentucky	Madison	Pyrimidal shaped the front of Roark building on EKU's campus	Robert Pace	SOTINKY43
Taxus cuspidata Sieb. & Zucc.	Kentucky	Madison	The Ravine on EKU's campus	Robert Pace	SOTINKY55

Microscopic Studies

Dried samples were processed via Scanning Electron Microscopy (SEM) (JEOL JCM-5000, Tokyo Japan) in high resolution mode at 10kV. A leaf, cone, pollen, or fruit are attached to metal disc via double-sided carbon tape. This disc is then inserted into the SEM, the chamber door closed, and evacuated of air. Investigation of the sample focused on the number of stomata present in the two bands on the underside of the leaves, along papillose cells locations, and cell structures of the leaf mid-vein. The distance across the stomatal bands were measured via SEM and this measurement was included in the subsequent images captured. Along with stomatal bands, the apices of each leaf were also recorded via SEM at various resolutions based on the leaf size. These uncompressed TIFF images were recorded at the highest resolution, and at magnifications of 270x and 330x for each sample. The TIFF image files were labeled with the sample number along with a letter of the alphabet based on whether it was leaf, flower, or fruit.

Dried herbarium specimens present problems when using a dissecting microscope, as the leaves of *Taxus* are prone to curling inwards towards the midvein. This curling can prevent an accurate numeration of the stomata within the stomatal bands. This curling of the leaves (revoluteness) also presents a problem due to the short depth of field found in most dissecting microscopes. Scanning Electron Microscopy (SEM) doesn’t suffer from shallow depth of field and offers an exemplary view of the *Taxus* abaxial leaf surfaces.

To combat the leaf curling, leaves were rehydrated with distilled water overnight and pressing the leaves in a flat state while drying. This will produce a flat subject that can then be easily explored via SEM or compound light microscope.

In addition to SEM microscopy, samples were also investigated using dissecting microscopes and compound microscopy. Dissection microscopes are useless in observation of key abaxial leaf structures due to the lack of proper light transmission through the leaves necessary to view stomatal bands. Compound light microscopes are capable of producing enough detail on the leaf abaxial surface as to provide appropriate count of stomata if the sample has been properly prepared as to flatten the leaves prior to inspection. Via SEM, the lower leaf surface was examined for characters such as marginal cell counts, cell surface morphology, number of stomata per band, apicular leaf morphology, bud scale morphology, and coloration. These characters were recorded and utilized to construct a taxonomic key to the *Taxus* species present within Kentucky.

Chemical Studies

The fresh samples collected in the field underwent chemical analysis via Direct Analysis in Real Time (DART) mass spectrometry utilizing a DART ion source (IonSense, DART® SVP, Saugus MA USA) coupled to a LTQ XL® linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) which was used to obtain the mass spectra of all the compounds analyzed using Xcalibur software (Thermo Scientific, San Jose, CA, USA).

The settings for the instrumentation were set to those found in (Banerjee et al. 2008). A deviation from the protocol found in Banerjee et al. (2008), is that no calli were used, instead the leaves and stem were sampled independently. A cotton swab saturated in ammonia was placed beneath the DART ion source, and re-wetted periodically during the chemical analysis. A few leaves were removed from the zip closed plastic bags via alcohol wiped forceps and subsequently placed into the ion stream of the DART ion source. These leaves were held in the ion stream for about a minute while the mass spectrometer recorded the spectrum. The recorded spectra were then saved to a file which was named after the sample number, followed by either the word "leaf" or "stem." The forceps were cleaned with an alcohol solution and dried with a kimwipe (Kimberly-Clarke, Kimwipe Irving TX) between each processed sample. Spectra were then subsequently normalized by excluding background readings both pre and post sample processing. The goal being to note presence and absence of five taxanes in order to characterize *Taxus* present within Kentucky.

After a fresh specimen is processed via DART mass spectrometry, it is then placed between a layer of newsprint, and that between blotter paper, and herbarium grade cardboard. These layers of cardboard, blotter paper, newsprint, and sample are placed in a plant press. The plant press that contains the fresh specimens are then air dried for a period of no less than three weeks. These now dried specimens are analyzed via Scanning Electron Microscopy, and then affixed to herbarium paper along with a label, for inclusion into the EKU herbarium.

Geographic Studies: GIS (Soils/Topography/Aspect/Slope)

The first step in generating a habitat suitability model for *Taxus canadensis* was to prepare a list of counties for which there were documented occurrences (specimen or literature report) for the species. This study utilized ArcGIS (ArcGIS 10.2.2, ESRI, Redlands CA) for habitat suitability modeling. This process begins by constructing a county map of Kentucky in which specimens are known to occur. For the habitat suitability modeling, the analysis required several ArcGIS data layers. Starting with Land Use / Land Cover provided by the National Land Cover Database for 2011. This is followed by Soil Surveys from the National Resource Conservation Services for 2014, then 10 M Digital Elevation Maps from USGS for 2013. Also added are the NHD Hydrology data layer for 2003. Then a slope raster is constructed using the DEM layer, followed by construction of an aspect raster layer using the DEM layer, concluded by the addition of Kentucky Department of Transportation's data layers for local and state roads.

The process of constructing the habitat modeling begins by examining the counties in which there are known populations of *Taxus canadensis* using the above data layers. Two features can immediately be realized as occurring within the counties that have known *Taxus* populations. First the parent soil type for these counties is classified as ultisols. The second observation is that the areas in which *Taxus* occur are mixed forests. With this in mind, the construction of the habitat suitability model begins. This process is begun by using ArcGIS's raster layer reclass/recode on the Land Use/Land Cover raster image, setting Mixed Forest as 3, with Evergreen Forest as 2, Deciduous Forest as 1, every other type is set to zero. Again use the raster reclass/recode on the soil survey layer, with ultisols set to 1, and all other soil types set to zero. Followed by buffering all roads based on their size. Interstates are buffered for 6 meters, highways by 5 meters, state roads by 4 meters, and local roads by 3 meters. The hydrology layer is also buffered based on the magnitude of the river or stream. Raster reclass/recode is then used on the slope raster layer based on sharp elevational changes being set to 3, with mild elevational changes set to 2, and no elevational changes set as 1. Raster reclass/recode is then used on the aspect data such that North and East are both set to 1, and South and West are both set to 2.

With all the data layers buffered and reclassed/recoded, ArcGIS's Map Algebra's Raster Calculator function is used to add the values from all the raster layers and store that data into a new raster data layer called "predictions". All the hydrology and road buffers are then combined into a single file using the Geospatial Analysis's Union command. What is left is a buffered layer and the "prediction" raster layer. The next task is to convert the buffered polygon layer to a raster layer using ArcGIS's conversion tool, assigning the raster layer a value of 0. ArcGIS's Map Algebra's Raster Calculator function is then used to multiply the buffer raster by the "prediction" raster layer, and output this raster layer to one called "FinalPredictions". The range of values now for this FinalPredictions layer data will be from zero to 9. The higher the positive value the more suitable the habitat is for *Taxus*.

CHAPTER IV

RESULTS

Morphology

In this study fresh specimens of *Taxus* were obtained for morphological and chemical comparisons from several populations (Table 2). Gross morphologies, such as features of phyllotaxy, leaf apices, and the angle of the petiole or bud to the stem, sometimes provide taxonomic differences. Most of these anatomical differences can be observed by the naked eye or via a 10× hand lens.

Leaf phyllotaxy is important in the gross morphology of *Taxus*. *Taxus* species can differ in their leaf density, leaf-ranking, along with whether the leaves present themselves upright or drooping. *Taxus* can differ significantly in how dense the leaves are arranged on the twig, along with the thickness of the twig. Some *Taxus* species employ an almost radial arrangement of leaves on the twig throughout the length, whereas others may be either two-rank, or radially arranged only on the branches apices.

Leaf apices vary between different *Taxus* species. *Taxus* can have blunt to quite sharp leaf tips. The species also can differ in the angle at the apices, some being acute, others obtuse. These features can be observed via tactile inspection along with the use of a hand lens.

Taxus share many gross morphological similarities between the individual species. This makes gross morphology alone incapable as a method of differentiation. Teasing the individual species apart from the *Taxus* complex requires the exploration, along with the gross morphologies, of the microstructures present on the leaves.

Structures of prime importance in distinguishing the different taxa of *Taxus* include; stomatal bands, papillosity, midvein cell structure, and leaf margins Dissecting microscopes can be employed to scrutinize these microstructures present on fresh specimens.

Growth Forms, Needles, and Seeds

Within Kentucky, there are only three species of *Taxus* that have been documented as occurring spontaneously (not cultivated); *Taxus baccata* (Figure 1), *Taxus canadensis* (Figure 2), and *Taxus cuspidata* (Figure 3). *Taxus canadensis* most often exhibits high leaf density, with leaves arranged mostly radially along the top half of the plant's body. The remaining lower body of *T. canadensis* possessing a more single ranking of leaves. *Taxus baccata* mostly exhibits an uplifting of needles in relation to the stem with lower needle density. *Taxus cuspidata* often exhibits only the newer growth having radial leaf arrangement, and single-ranked for the remainder of the branches.

The reproductive cones, particularly the female cones of *Taxus* (Figure 4) can vary between species in both size and shape. Unfortunately availability of seeds from herbarium specimens and field collections prevent elucidating species via seeds within the scope of this research. For the few seeds available, it was noted that some seeds were latitudinally spherical, and some being longitudinally angled to the point of being veritably lobed.

Male cones (Figure 5) are easily distinguished from the female due to more knobby or cauliflower like structure. The male cones erupt in early spring producing copious amounts of golden pollen.

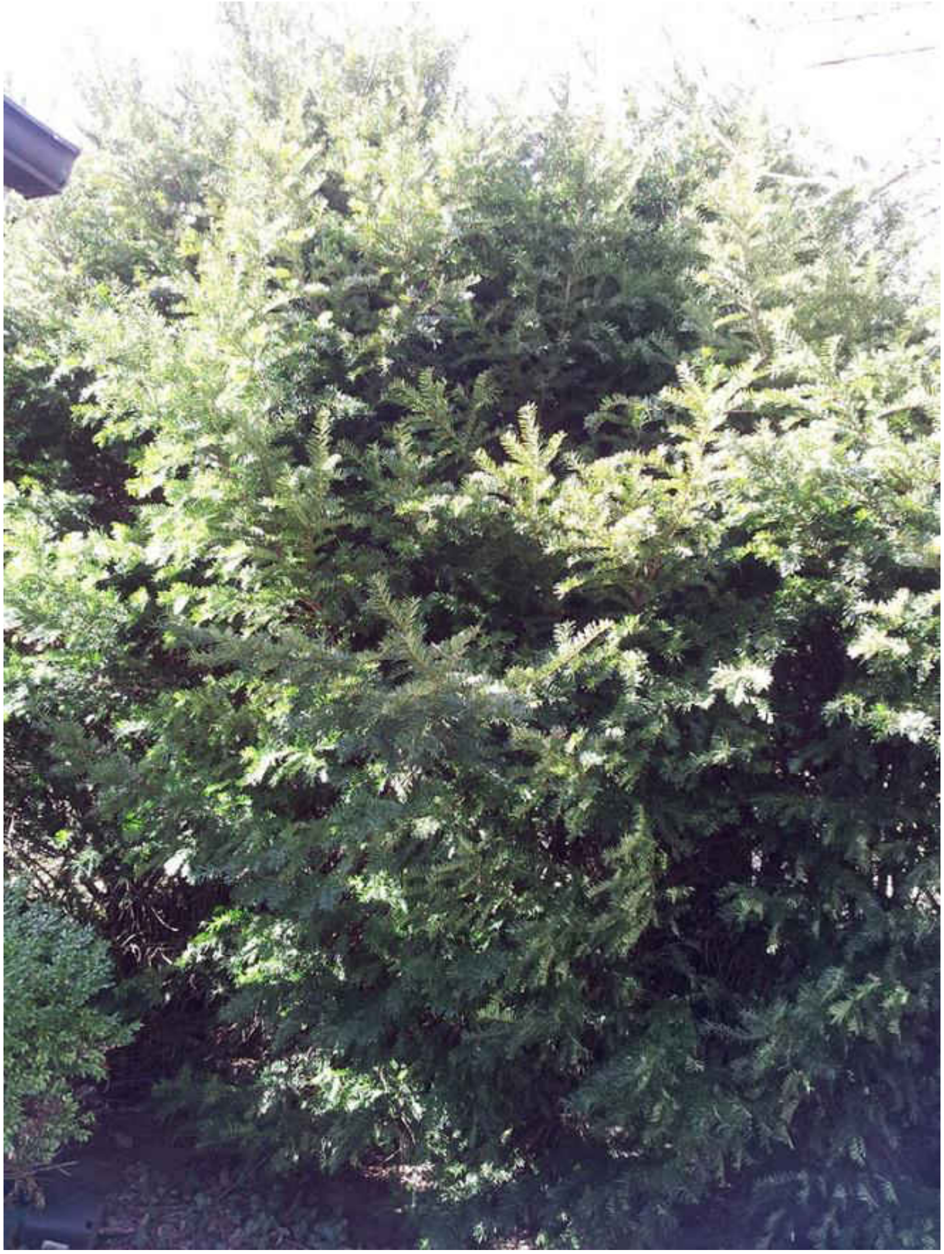

Figure 1. Multi-stemmed *Taxus baccata* ca. 4 m tall, located in a back yard, sample # SOTINKY01.

Figure 2. *Taxus canadensis* producing a low prostrate ground cover under 1 m tall, sample # SOTINKY26.

Figure 3. Single stemmed *Taxus cuspidata* ~ 12 m tall located on Eastern Kentucky University's campus outside the Miller Building surrounded by multi-stemmed *Taxus* × *media* hedges ~ 2 m tall.

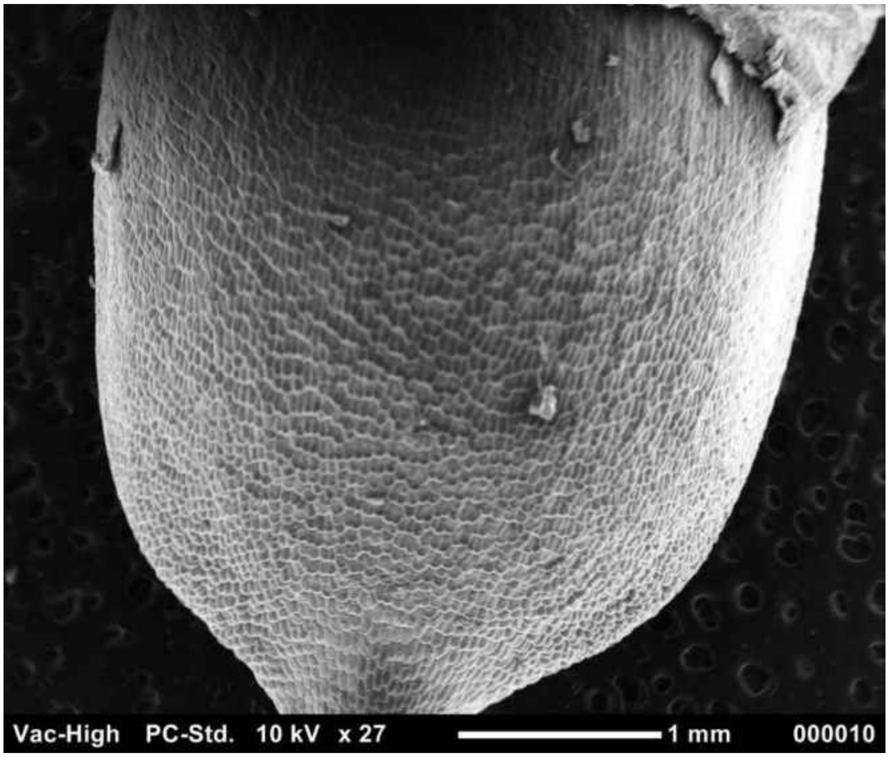

Figure 4. SEM image of a mature female cone of *Taxus baccata*, sample # SOTINKY01.

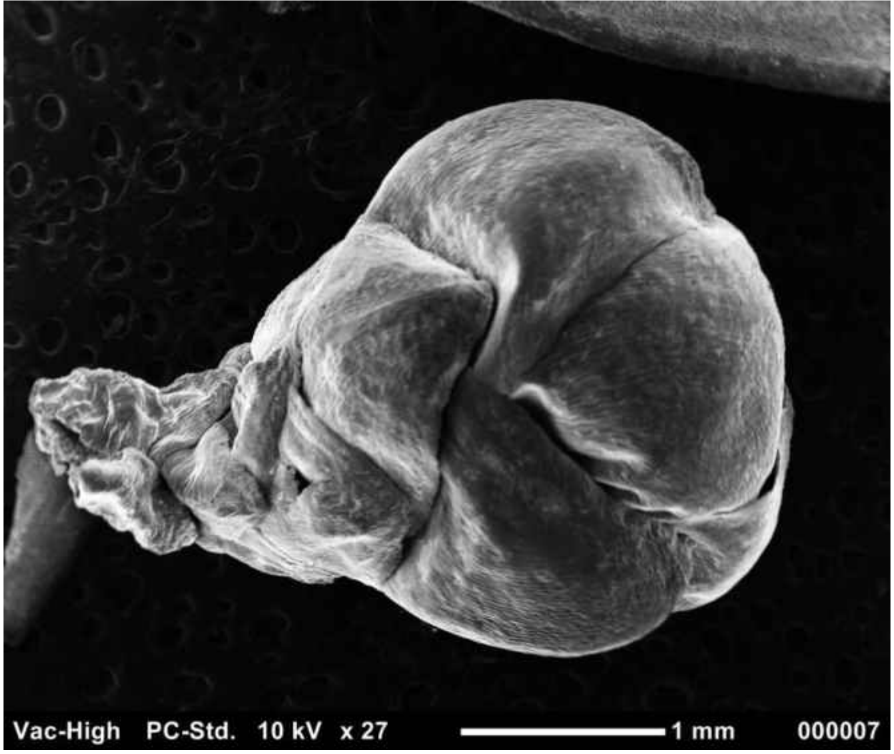

Figure 5. SEM image of an immature male cone of *Taxus* × *media*, sample # SOTINKY02.

With *Taxus*, stomates are found in two distinct bands on the leaf abaxial surface. These stomatal bands are bisected by the longitudinal leaf midvein. The number of stomates present within these bands is highly valuable in identification of the taxa. The number of stomates within these bands is not exact for each taxa, and varies subtly at the population level within constraints.

Analysis of the SEM images (Figure 6–Figure 17) in Appendix B for both native and non-native *Taxus* species show that the number of stomata found within the stomatal banding is an easy way to determine if a specimen is native or non-native to Kentucky. *Taxus canadensis*, our only native species will always display less than 6 stomates per band (Figure 6), whereas non-natives will possess 6 or more stomates per band. A key to *Taxus* species is presented after the genus descriptions that will allow circumscription of *Taxus* species within Kentucky with the exception of *Taxus* × *media* which is too variable in microstructures as to differentiate.

Stomatal banding alone will only permit determination of whether a specimen is native or non-native. In order to provide circumscription it is necessary to examine a variety of microstructures such as abaxial leaf surface papillosity. Within select species of *Taxus*, there will be small surface cells that exhibit an elevated almost conical projection of the cell membrane. The location of these projections within the cell, and their occurrence pattern on the leaf abaxial surface is invaluable.

The shape and texture of the midvein are also important features that are necessary for correct identification to the species level. *Taxus* differ in the size, cell geometry, and papillosity along the midvein.

Leaf margins are also valuable in teasing out the individual species of *Taxus*. Leaf margins can exhibit different morphologies, with some species having different levels of margins being revolute vs. straight leaf margins.

Chemistry

During the chemical analysis the goal is to quantify the relative intensity of five taxanes (Figure 18–Figure 22) within 21 samples of fresh *Taxus* cuttings. The taxanes, without the dopant ammonium hydroxide, we were looking for are *m/z* of 504 (Figure 18), *m/z* of 518 (Figure 19), *m/z* of 532 (Figure 20), *m/z* of 546 (Figure 21), and *m/z* of 562 (Figure 22). Ammonium hydroxide is used as a dopant similar to the study by Baneerjee et al. (2008), it is expected that the mass to charge ratios to be increased by *m/z* 18. Thus it is expected that the taxanes will show up via mass spectrometry as *m/z* 522 $[M + NH_4]^+$ (Figure 18), *m/z* 536 $[M + NH_4]^+$ (Figure 19), *m/z* 550 $[M + NH_4]^+$ (Figure 20), *m/z* 564 $[M + NH_4]^+$ (Figure 21), and *m/z* 580 $[M + NH_4]^+$ (Figure 22) respectively (see Appendix C). One goal of this application of DART M.S. would be to characterize species of *Taxus* via the presence or absence of these five taxanes. This could lead to a chemical method of differentiating species. In this study the relative intensity of these five taxanes differed dramatically between the stem and leaves of the same plant. Eleven samples had no detectable amount of the five taxanes we investigated (Table 3 and Figure 23–Figure 42 in Appendix D). Of the ten samples that did show the presence of the taxanes, the predominantly present taxanes had the molecular weight of 536m/z. No sample showed the presence of all five taxanes. (Baneerjee et al. 2008) had higher relative intensity for the same five taxanes with their study possibly because they were using the calli of *Taxus* plants, not leaves and stems. The wounds used to produce such calli could increase anti-herbivory compounds such as taxanes within the plant by simulating herbivory.

It may be possible to characterize *Taxus* via DART M.S. using a more sensitive detector, measuring taxanes content of calli, and looking for more taxanes than five. A question that needs to be explored is whether hybrid species of *Taxus* vary in taxanes production from their parents. Considering the wide variation in phenotypic differences possible between the same hybrid species, it may be impossible to differentiate hybrid species from other taxa.

The findings of this study suggest that utilizing leaf and stem alone to characterize native and non-native Taxus species via DART was not feasible using only five taxanes. Detection of taxanes from leaf and stem was hampered by their extremely low concentrations of the taxanes present within these tissues. A potential problem with using DART MS alone is that there could be many other molecules of the same molecular weight as taxanes. These other molecules can saturate the sensor reducing the detection of taxanes. One solution for this issue would be to use HPLC or UPLC to separate out as many non-taxanes from the plant material as possible. Another complication in this analysis was due to the use of only five taxanes, with a larger number of taxanes being explored success may be possible. Utilizing wound calli tissues for the analysis isn’t practical for the characterization of species due the amount of time and effort needed to obtain results. Chemical fingerprints were produced via DART MS, but they were not unique to each *Taxus* species.

Table 3. List of taxanes present per sample and corresponding figure. Letters after Sample # refer to whether sample was a leaf (L) or stem (S).

Figures	**Sample#**	**Taxanes Present**
Figure 23	SOTINKY01L	$[M + NH_4]^+$ *m/z* 536 m/z
Figure 24	SOTINKY01S	None
Figure 25	SOTINKY02L	None
Figure 26	SOTINKY02S	$[M + NH_4]^+$ *m/z* 536 m/z
Figure 27	SOTINKY03L	None
Figure 28	SOTINKY03S	None
Figure 29	SOTINKY07L	$[M + NH_4]^+$ *m/z* 522, $[M + NH_4]^+$ *m/z* 536
Figure 30	SOTINKY07S	$[M + NH_4]^+$ *m/z* 522, $[M + NH_4]^+$ *m/z* 536
Figure 31	SOTINKY08L	$[M + NH_4]^+$ *m/z* 536
Figure 32	SOTINKY08S	None
Figure 33	SOTINKY33L	$[M + NH_4]^+$ *m/z* 536, $[M + NH_4]^+$ *m/z* 550, $[M + NH_4]^+$ *m/z* 564
Figure 34	SOTINKY33S	$[M + NH_4]^+$ *m/z* 536
Figure 35	SOTINKY36L	None
Figure 36	SOTINKY36S	$[M + NH_4]^+$ *m/z* 536
Figure 37	SOTINKY42L	$[M + NH_4]^+$ *m/z* 536
Figure 38	SOTINKY42S	$[M + NH_4]^+$ *m/z* 536
Figure 39	SOTINKY53L	$[M + NH_4]^+$ *m/z* 536
Figure 40	SOTINKY53S	$[M + NH_4]^+$ *m/z* 522, $[M + NH_4]^+$ *m/z* 536 , $[M + NH_4]^+$ *m/z* 550, $[M + NH_4]^+$ *m/z* 580
Figure 41	SOTINKY55L	$[M + NH_4]^+$ *m/z* 536, $[M + NH_4]^+$ *m/z* 550
Figure 42	SOTINKY55S	$[M + NH_4]^+$ *m/z* 522, $[M + NH_4]^+$ *m/z* 536, $[M + NH_4]^+$ *m/z* 550

Habitat Model: Known & Potential Occurrences

Several maps were generated, including maps to show the location of various *Taxus* species within the continental U.S. (Figure 43) and a county map of known occurrences of *Taxus canadensis* within Kentucky (Figure 44). Maps were also generated to show important environmental associations associated with *Taxus canadensis* within Kentucky (Figure 45 & Figure 46), along with maps of known and predicted areas within the state (Figure 47–Figure 49). Maps were also created that show the predicted areas within counties with known populations of *T. canadensis* and counties with suggested potential populations. All maps are in Appendix E. The following counties are presented in two groups, those counties that have documented records, and those in which the GIS model predicted possible occurrences:

1) Counties with Known Occurrences:

Carter County – GIS modeling predicts the best areas for *Taxus* within the county to be within the southern regions with slight emphasis on the south central (Figure 50).

Jackson County - GIS modeling predicts the best area for *Taxus* within the county to be wide spread and patchy, with slight emphasis on the western and central regions of the county (Figure 51).

Menifee County – GIS modeling predicts the best area for *Taxus* within the county to be within the southern half of the state and to a lesser degree the eastern half of the county (Figure 52).

Powell County - GIS modeling predicts the best area for *Taxus* within the county to be mostly in the western part of the county and to a lesser degree in the southern portion of the county (Figure 53).

Pulaski County - GIS modeling predicts the best area for *Taxus* within the county to be along the eastern region, close to the county boundary (Figure 54).

Rowan County – GIS modeling predicts the best areas for *Taxus* within the county to be within the southeastern region (Figure 55).

Wolfe County – GIS modeling predicts the best area for *Taxus* within the county to be within western half of the county (Figure 56).

2) Counties with GIS Model predicted occurrences:

Elliott County – GIS modeling predicts the best area for *Taxus* within the county to be in the western region of the county and to a lesser degree the northern portion of the county (Figure 57).

Laurel County – GIS modeling predicts the best area for *Taxus* within the county to western regions of the county (Figure 58).

Lee County – GIS modeling predicts the best area for *Taxus* within the county to be north eastern quarter of the county (Figure 59).

McCreary County – GIS modeling predicts the best area for *Taxus* within the county to mostly found in the eastern half of the county (Figure 60).

Morgan County – GIS modeling predicts the best area for *Taxus* within the county to be within the northern and western regions of the county (Figure 61).

Whitley County – GIS modeling predicts the best area for *Taxus* within the county to be in the north western regions, and to a lesser degree the extreme southwestern area of the county (Figure 62).

Genus Description

Taxus Linnaeus, Sp. Pl. 2: 1040. 1753; Gen. Pl. ed. 5, 462, 1754.

Yew

Trees or shrubs of various sizes, mostly dioecious (monoecious in *T. canadensis*). Bark thin, rusty brown, and peeling. Branches highly variable and can be ascending, prostrate, or drooping; twigs alternate, most being isodichotomously branched, young twigs being paler green, older twigs exhibiting a darker green to almost red coloration with age. Leaves vary between being 2-ranked on older or shaded twigs, and almost spirally arranged on younger or sun exposed twigs, springy to stiff in texture; leaves tapering to short petiolar base, midrib continuous from petiole to apex, and decurrent on the stem. Abaxial leaf surfaces having outer marginal cells, two stomatal bands, and the midrib. Stomatal bands fainter in color to surrounding cells, with various degrees of papillosity; leaf apex mucronate and varying between soft to stiff. Pollen cones globose, with cauliflower like texture, beige to honey-colored, and bearing golden anemophilous pollen. Cone with single seed, terete to triangular, surrounded by a fleshy cup-shaped fleshy aril. Seed falling in late fall into early winter.

Species number highly variable with Hils (1993) suggesting 6 to 10, and Spjut (2007) suggesting as many as 24 (representative species are shown in Figure 63–Figure 71 in Appendix F), most occurring in a Holarctic distribution.

Keys to Species

1. Plant a small shrub, under 2 m tall, typically monecious; needles to 3 cm long (sometimes longer in older plants), with 2 bands of stomates on lower surfaces, each band usually 5 to 7 stomates wide; papillae obscure between stomates …….. **T. canadensis**

1. Plant a shrub or small tree, often over 2 m tall (to 25 m), mostly dioecious; needles to 4 cm long, with two bands of stomates on lower surfaces, each band 7 or more stomates wide; papillae prominent between stomates.

    2. Plant fastigiate, columnar, or pyramidal in commonly cultivated form; leaves with 8 to 10 stomates per band on densely papillose abaxial leaf surface, stomatal band olive green…………………………...…………………. **T. baccata**

    2. Plant pyramidal or as wide as tall; leaves with 11 to 13 stomates per band on abaxial leaf surface, stomatal band yellow-green …………...… **T. cuspidata**

Species Accounts

1. *Taxus canadensis* Marshall. (Figure 65).

Canada yew, American yew.

Synonyms: *Taxus baccata* Linnaeus subsp. *canadensis* (Marshall) Pilger; *T. baccata* var. *minor* Michaux; *T. minor* (Michaux) Britton; *T. procumbens* Loddiges

Shrubs under 2 m, mostly monoecious, low, dichotomously branched, patchy, spreading to prostrate. Bark reddish brown, very thin. Branches prostrate and ascending. Leaves up to 2.5 cm, dark green on axially, and pale green abaxially, stomatal band showing light papillosity with 5 to 7 stomates per band. Seed angular in shape under 5 mm in diameter, $2n = 24$ (Dark 1932, Khoshoo 1960).

Cones appearing early spring and seeds maturing in late summer to early fall. Understory short shrub, either patchy in distribution or as a groundcover, occurring in both deciduous and evergreen forests. Often found in Kentucky along rocky cliffs, creek banks, and cave openings.

Native to Kentucky, distribution is from Ontario to Quebec and south to Tennessee and North Carolina.

Documented counties in Kentucky: Carter (Meijer, September 1972 KY), Jackson (Jones 3965, EKY), Menifee (Huie-Netting 50, EKY), Powell (Jones 8111e, EKY), Pulaski (Denham 8/28/1985, EKY), Rowan (Risk 11-403, KNK), and Wolfe (Thieret 08/16/1982, KNK). No voucher record was located to document the occurrence of the species in Lee County, as mapped by Medley (1993) and Campbell and Medley (2014), or Owsley County, as mentioned by Gonsoulin (1975).

The literature report by Hussey (1876) from Edmonson/Barren county area could not be verified. These regions are out of the known range of the species, but still possible, as there are disjunct populations of mixed mesophytic species still found in the area (Jones 2005).

2. *Taxus baccata* L. (Figure 63)

English yew, European yew

Tree reaching heights of 20 m, often with crisscrossing branches. Leaves up to 4 cm in length, stomates occurring in 8 to 10 per band, mostly papillose between marginal cells and olive green stomatal bands.

Cones appearing early spring and seeds maturing in late summer to early fall. Seeds are terete and under 5 mm in diameter, 2n = 24 (Dark 1932, Khoshoo 1960).

Native to Western Europe, not native to Kentucky and escaping infrequently.

Documented counties within Kentucky: Whitley (Weckman 10778, EKY).

3. *Taxus cuspidata* Siebold and Zuccarini (Figure 66)

Japanese Yew, Rigid Branched Yew

Tree or shrub up to 18 m, with stout stems often with short recurved branches. Leaves up to 3 cm in length, stomatal band greenish yellow in color, with 11 to 13 interrupted stomates per band, mostly papillose in bands within marginal cells.

Cones appearing early spring and seeds maturing in late summer to early fall. Seeds are quadrangle near apex ~5 mm in diameter, 2n = 24 (Dark 1932, Khoshoo 1960).

Native to Japan and Korea, not native in Kentucky, and escaping infrequently.

Documented counties within Kentucky: Jefferson County (Medley and Thomas 18282-87, as noted in Medley, 1993; specimen could not be located, but is likely in storage at Western Kentucky University). Other herbarium records for this species are apparently from cultivated specimens.

CHAPTER V

DISCUSSION

Macroscopic/Microscopic Investigation

A large problem that has hampered an understanding of *Taxus* has been the difficult nature of distinguishing the taxa. An accurate and detailed taxonomic key is needed, but difficult to construct, due to the great similarity among taxa in gross morphology. It is therefore best to utilize both macroscopic and microscopic characteristics in comparing the taxa. For the macroscopic investigation, the focus should be on branching patterns, growth patterns, and the presence or absence of persistent bud scales. The microscopic investigation should center on small structures on the abaxial surface of the leaves, looking for the arrangement and number of stomates found within the bands, along with the density and location of any papillae. Utilizing macroscopic features alone, it is possible to distinguish Kentucky's native *Taxus canadensis* from mature non-native species. The microscopic features are impossible to avoid when it comes to circumscription of the non-native species. The combination of macroscopic and microscopic features allows for the construction of a key to more accurately identify the native and non-native species in Kentucky, as indicated in the key to species above.

Chemical Analysis

Intense chemical analysis with the aim of differentiation of species of *Taxus* undertaken with this research was aimed at reducing the amount of plant matter necessary needed to characterize a particular *Taxus* species. Unfortunately, *Taxus canadensis* is threatened in Kentucky and exists only as a small shrub, and this has to be taken into consideration when harvesting plant material. Kentucky's populations of *Taxus canadensis* are simply too fragile to support harvesting of entire plants for the sake of identification via their chemistry. These plants use taxanes to deter herbivory and as an insecticide. Plants often will vary in the amount of taxanes. Plants in general tend to not produce abundant anti-herbivory/insecticide compounds without having experienced being browsed by herbivores or insects. To produce abundant anti-herbivory/insecticide compounds otherwise would be a waste of cellular energy. As such the chemical abundance of taxanes within the plant would naturally be expected to vary based on things like environment, particularly climate, soil chemistry, hydration, and location. It is also worth noting that taxanes are large and energy expensive molecules, and are generally quite toxic to most mammals with the exception of cervids (Conover & Kania 1988, Windels & Flaspohler 2011). *Taxus* in general produce very small amounts of these taxanes, and is one of the reasons for the decline of *Taxus brevifolia* on the West coast, due to the amount of bark/needles harvested in order to produce a small amount of the chemotherapeutic drug Paclitaxel. Sophisticated ion detectors in modern mass spectrometers can have a problem detecting these compounds in raw plant matter due to extremely low concentrations present in the plant materials. More plant material can obviously be harvested from species that reach larger sizes, compared to the shrubby *Taxus canadensis*. Another possibility is to separate out the other compounds present within the plant matter, thus producing a more concentrated extract to be processed via DART Mass Spectrometry. This has been traditionally handled via coupling of Mass

Spectrometry with HPLC. This project in hindsight could benefit from using far more taxanes in the characterization in order to have potential success.

GIS Modeling

Another problem with studying living *Taxus* within Kentucky is locating the populations. Kentucky has vast areas of forest within the plant's habitat with considerable changes in elevation that makes traversing its habitat challenging. With *Taxus canadensis* preferring steep slopes and deep valleys, it is naturally secluded. *Taxus canadensis* also has a very confined distribution within Kentucky, and understanding why it inhabits the region it does and is absent from others hasn't been understood. Another difficulty in finding *Taxus canadensis* populations in the wild is that it often grows in close proximity to other conifers, particularly the Eastern Hemlock (*Tsuga canadensis*), which looks from a distance quite similar to *Taxus canadensis*. This often forces the use of binoculars for scouting and requires close proximity in order to obtain a positive identification as a yew. The rugged terrain inhabited by *Taxus canadensis* may result in the species being under-collected and under-represented in local herbaria.

The difficulty in finding *Taxus* in Kentucky provides a rationale for habitat modeling. By examining details such as soil, climate, slope, elevation, hydrology, land use, land cover, and aspect, one can better understand the habitat needs for the plant. These type models provide an understanding of the plants distribution in context of the environment. Models also permit making predictions as to where the plant could potentially be found. This allows an individual to focus on areas in which the model predicts a higher likelihood of finding the plant.

The modeling for *Taxus canadensis* within Kentucky aims to provide three main types of maps. One series of maps generated shows the spatial arrangement of various aspects of habitat that are associated with *Taxus*, such as ultisols, mixed forest types, and land use/land cover. Another series of maps shows predictive hot spots in which the model indicated elevated potential of occurrence for counties with known populations of *Taxus canadensis*. The final series of maps contain predictive hot spots for counties in which there were no specimens available. Predictive modeling for these counties could be useful in providing new county records for *Taxus canadensis*.

This modeling of *Taxus canadensis* in Kentucky provides a basic framework for further study of *Taxus* by assisting future researchers in locating *Taxus* populations. As the knowledge of *Taxus* increases over time, the modeling can be improved, providing more accurate predictions. Modeling also could be used to show how global climate change could affect distributions of *Taxus canadensis*.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The species *Taxus canadensis* within Kentucky has an unusual distribution pattern that is influenced by soils, forest types, disturbance regimes, and climate. This research explored soils present within the native range in Kentucky, along with elevation, forest type, hydrology, and aspect in order to model predicted habitat for this species. This modeling will assist future studies involving *Taxus canadensis* within the state, and will act as a base for more sophisticated modeling. Three main types of maps were produced from this research. The first series of maps show distribution of soils and forest type that are associated with this species. The second series of maps, shows habitat modeling for *Taxus canadensis* in counties where populations have been previously located. The third series of maps shows habitat modeling for *Taxus canadensis* in counties where there are no records of occurrence.

Yews are heavily employed by landscapers and are a horticultural favorite for hedges and topiaries. This research explored whether these cultivated *Taxus* could potentially escape into the wild. The findings show that escape is rare, and is confined to *Taxus cuspidata* and *Taxus baccata*. The most popularly cultivated non-native is *Taxus* × *media* and has not been recorded as escaping into the wild.

A major obstacle in *Taxus* research is difficulty in identification. Mistakes in identification can invalidate any information gathered about a species. This research explored the morphological differences between native and non-native species of *Taxus* that are found in the wild areas of Kentucky. A variety of equipment was employed in morphological examination including scanning electron microscope (SEM). A product of the detailed examination was the production of a dichotomous key for *Taxus* species based on both macroscopic and microscopic features.

Another means of identification of *Taxus* species was explored using chemical characters instead of morphological ones. This research involves the observance of five taxanes within a variety of *Taxus* species utilizing Direct Analysis in Real Time Mass Spectrometry (DART MS). This technique of identification via the presence and absence of taxanes was not successful due to the extremely low amount of taxanes present within the samples, and due to the high variability in concentration of these taxanes due to season and herbivory.

The future for native *Taxus canadensis* within Kentucky could be affected by global climate change. This could be expected to alter the environment in Kentucky in ways that could provide a less hospitable environment for the species. *Taxus canadensis* prefer cooler and wetter climate. If the climate were to shift towards warmer or drier season this could increase the frequency of forest fires and drought, which would be highly detrimental to this relict of glacial times.

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APPENDICES

APPENDIX A: Specimen Citations

1. Taxus baccata Linnaeus.

India:

N/A. N/A County — Deoban, Chakrata, UP., J. Sayup (WVA);

U.S.A.:

CALIFORNIA. Los Angeles County— f, Don A. Emerson (WVA); Yolo County—Animal Science Building entrance, UCD, Pat Sullivan (WVA);

KENTUCKY. Fayette County — University of Kentucky Campus, J. L. Gentry 1140 (KY); Madison County — backyard of 203 Moberly Ave, Robert R. Pace SOTINKY01 (EKY); Whitley County—Woods edge, Resort Cabin Road just off Hwy 90; ca 0.1 mi SW of Dupont Lodge Loop Rd, Cumberland Falls State Park., Timothy J. Weckman 10778 (EKY);

NEW YORK. Bronx County—New York Botanical Garden, Bronx Park, Bronx Co., New York City, N. Y., Harold N. Moldenke 4310 (WVA);

OHIO. Wayne County—Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A30-75 (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A30-194 (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret B01-086 (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret BA31-64 (KNK);

2. ***Taxus brevifolia*** Nuttall

U.S.A.:

CALIFORNIA. Siskiyou County—, A. T. Leiser D 2504 (WVA);

IDAHO. Idaho County—Several miles NE of Lowell along US highway 12., Matthew H. Hils 1036 (KNK); Ca. 4 miles SW of Lolo Pass (border between Idaho and Montana) ca. 60 air miles NE of Lowell., John W. Thieret 56101 (KNK); Devoto Memorial Cedar Stand near Lolo Pass., Michael Wade (WVA); Latah County—Along Mannering Creek, 2 miles south of Benewah County line, St. Joe National Forest., Marion Ownbey 2020 (WVA); Soshone County—Near Avery, Wendel Swank (WVA); Benton County—Small tree in cultivation, Corvallis, Jeffrey G. Thieret (KNK); Jackson

County—Elevation 4700 ft in Cascade Range. Ca 20 miles E of Medford along highway 140, Matthew H. Hils (KNK);

OREGON. Jackson County—Prospect District Near the Rogue River, Elevation 2,700', R. J. Mastrogiuseppe (WVA); Josephine County—Ca. 4 air mi W of Selma - Siskiyou National Forest - Along Snailback Creek - N of Illinois River Road (Natioal Forest Service Road 4103)., Robert F. C. Naczi 3243 (KNK); Klamatk County—Al Sarena Buzzard Mine, Rogue River National Forest, R. J. Mastrogiuseppe (WVA); Multnomah County—Hoyt Arboretum - Portland., Matthew H. Hils 1053 (KNK); N/A County — Northwestern Oregon, M. L. Bransen (EKY); Parkdale County—, Ralph W. Mohr 119 (KY); Unknown County—Willamette National Forest, Richard J. Obyc (WVA);

WASHINGTON. Chelan County—Red Mt. Mine, Don Cole (WVA); Red Mt. Mine, Don Cole (WVA); Red Mt. Mine, Don Cole (WVA); Kittitas County—Along Cle Elum River near Davis Mt. trail bridge. Elevation 2500', Donald Cole (WVA); Lewis County—Snoqualmie National Forest Near Mineral., Robert E. Henderson 18 (WVA); Pierce County—Mount Ranier National Park, Longmire Meadows, El = 2700', George A. Hall H-120 (WVA); Thurston County—At the head of Mud Bay, F. G. Meyer 1589 (WVA);

3. ***Taxus canadensis*** **Marshall**

Canada:

Quebec. Terrebonne County—St-Jerome, Leg. Frere Rolland Germain 8362 (WVA);

U.S.A.:

CONNECTICUT. Tolland County—Route 15, Union, near Mass. Line, G. B. Rossbach (WVA);

ILLINOIS. Carroll County—, Robert A. Evers 108298 (KNK); Daviess County—Apple River Canyon State Park, Alfred C. Koelling 396 (TENN);

INDIANA. Monroe County—In Bloomington Indiana, R. Dale Thomas 123084 (TENN);

KENTUCKY. Carter County—Carter Cave State Park, W. Meijer (KY); Cascade caverns, F. A. Gilbert 831 (WVA);— Cascade Caverns, F. A. Gilbert 831 (EKY); Above Tygarts Creek, upstream from bridge on KY 182 near entrance to Carter Caves State Park., Timothy J. Weckman 1182 (EKY); Ky 182 at S end of bridge over Tygarts Creek., Elizabeth M. Browne 9601 (EKY); Jackson County—Along War Fork below Resurgence Cave, Julian Campbell (KY); Along Warfork Creek., Ronald L. Jones

3965 (EKY); 1.1 miles SE of Wind Cave Church, on the south side of War Fork Creek, 60' above the creek at entrance to War Fork Cave, about 8 air miles northeast of McKee, Greg Sievert (EKY); Menifee County—Daniel Boone Natioal Forest. Middle Fork Red River - N Bank ca 2 mi upstream from bridge of KY. 715 over Red River, Alvin Mosley 1 (KY);Frenchburg; Gladie Creek - Central section limestone talus, Julian Campbell (KY); Roadside along KY 715, Approx. 3mi Wolf/Menifee Co. boarde at bridge., Kathryn Huie-Netting 50 (EKY); Both sides of Gladie Creek above 1,000 ft on either side of dry fork mough and along Dry Fork about 1,000 ft above mouth., J. Campbell (EKY); Powell County—Along HW11, streamban just south of entrance to Natural Bridge State Park, near Wolfe County line., Ronald L. Jones 8111e (EKY); Pulaski County—White oak creek off Rt 196., Andy Denham (EKY); Rowan County—unnamed tributary of Minor Cr.- 0.3 mile north of Minor Cr. - Shop Br. Jct., Allen C. Risk 11-403 (KNK); Minor creek tributary 0.4 miles N of Shop Branch, Julian Campbell (KY); Wolfe County—South of Bridge and on West side of road. Ca. 1.7 mile south of Wolfe-Powell Co. Line on Kentucky highway 11., John W. Thieret (KNK); Daniel Boone National Forest. KY. 11 - 1.7 mi. S of Wolfe-Powell Co. Line., Robert Brooks (KY); On KY 11, two miles south of Natural Bridge State Park., Jennifer R. Francis 41 (EKY); Cliffs above KY 715 about 0.6 road mile northwest of bridge over Red River, in ravine north of Sky Bridge., Bryce D. Fields 881 (EKY); Along Middle Fork Red River, Robert R. Pace SOTINKY26 (EKY); Along Middle Fork Red River, Robert R. Pace SOTINKY27 (EKY); Along Middle Fork Red River, Robert R. Pace SOTINKY28 (EKY); Along Middle Fork Red River, Robert R. Pace SOTINKY29 (EKY);

MASSACHUSETTES. Worcester County—on W side of Hwy. 202 about 300 meters. Just N. of the village of Winchester Springs, Vernon Bates 247 (TENN);

MAINE. Waldo County—Meguntioook Lake, Ray C. Friesner 9067 (WVA);

MICHIGAN. Alger County—Pictured Rocks National Lakeshore. Ca. 10 miles NE of Munising., John W. Thieret (KNK); Miner's Falls, James C. Myers 209 (WVA); Cheboygan County—George along Brutus Road, James C. Myers (WVA); Chippewa County—Sugar Island, Sault Ste. Marie, Mrs. Oscar Lund (WVA); Emmet County—, John W. Thieret 48674 (KNK); Luce County—Along Sucker River, highway 416 ca. 0.6 miles SE of jct 416/H58; east of Grand Marais, MI., Timothy J. Weckman 5951 (EKY); Mackinac County—Cut River Gorge - crossing of US Route 2, George A. Hall H-66 (WVA); Ontonagon County—Behind Mineral River Shopping Plaza, White Pine, Michigan, Timothy J. Weckman 4251 (EKY);

MINNESOTA. Fillmore County—, John W. Thieret (KNK); Lake County—Cascade River Gorge, Ken E. Rogers 10073 (TENN); St. Louis County—T 61N R 21W NW 1/4 of SW 1/4 of Sec 34, Roger Lake 98-6 (WVA);

N/A. N/A County—NE x, Sec 27, T 14 N, R 4 W CMU Campus, Bruce P, Beerbower (WVA);

NORTH CAROLINA. Ashe County—Along Long Hope Creek at the Ashe-Watauga County line on Richardson Farm. First reported in Wauauga county by Gladston McDowell and Dr. J. H. Hardin., S. W. Leonard 2099 (TENN); Along Long Hope Creek at the Ashe-Watauga County line on Richardson Farm. First reported in Wautauga county by Gladston McDowell and Dr. J. H. Hardin., S. W. Leonard 2099 (TENN); Along Hope Creek at the Ashe-Watauga County line on Richardson Farm, S. W. Leonard 2099 (KY); Along Long Hope Creek at the Ashe-Watauga County line on Richardson Farm, S. W. Leonard 2099 (WVA);

NEW HAMPSHIRE. Belknap County—Rt. 107, near boundary Pittsfield., L. E. Richardson 6044 (WVA); Cheshire County—SW corner of intersection of Hwy. 9 and Hwy. 123. Twon of Stoddard. 430 meters, Vernon Bates 46 (TENN); Glebe Road., H. E. Ahles 76358 (WVA);

NEW YORK. Dutchess County—Three miles east of Red Hook, Hays Helmick (WVA); Erie County—800 ft. N of sewer access road, 900 ft. E of Meadow Drive, David D. Taylor 70 (WVA); 800 ft. N. of sewer access road, 900 ft. E. of Meadow Drive, David D. Taylor 70 (EKY); N/A County—, (WVA); Oneida County—W. bank of E. Branch of Fish Creek, Yorkland Rd., Anneville., G. B. Rossbach 10506 (WVA); Tomkins County—Six Mile Cr., Ithaca N.Y., Mr. H. A. Davis 1645 (WVA); Warren County—Along road S. of pond, A. S. Margolin 128 (WVA);

OHIO. Licking County—Near Fallsburg Sec. 8, Peg Heimbrook (WVA); Ottawa County—, Ronald L. Stuckey 4839 (KNK); Wayne County—Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret (KNK);

PENNSYLVANIA. Bucks County—Rich, wooded cliffs along Tchickon Creek, 1 mi. S.W. of Ottsville, J. W. Adams 8334 (WVA); Erie County—Six Mile Creek Park-E side of Erie - along Six Mile Creek 150 ft upstream from Depot Rd (Hwy 531) bridge on S side of creek., Dwayne Estes 11566 (TENN); Erie County—Elk Cr, 102mi W. of bridge on Rt 98, L. K. Henry (WVA); Bog along Hubbel Run, 3.5 mi. SE of Wattsburg, L. K. Henry (WVA); Tioga County – South of Willsboro, E. M. Gress (WVA); Picket County—, G. Gonsoulin 4332 (KNK);

TENNESSEE. Pickett County—Rock Creek Trail on northern slope approximately 1.5 miles east of junction of Rock Creek and TN 154., Joey Shaw JSh# 762 (TENN); Approximately 1.5 miles east of junction of Rock Creekand TN 154, Joey Shaw JSh# 762 (EKY); Washington County—Veterans Administration Center, Mountain Home, J. M. Roland (TENN);

VIRGINIA. Montgomery County—Above Tom's Creek at Summyside School House, 10 miles N.W. of Blacksburg., A. B. Massey 5067 (WVA); Prince William County—Population growing on steep northeast-facing shaly slope in mixed woods above southwest side of Bull Run, between Manssas National Battlefield Park and gas pipeline, elevation c. 180 ft., P. M. Mazzeo; W. W. Diehl 2,772 (WVA); Smyth County—Altitude 2075 feet, John K. Small (WVA);

VERMONT. Essex County—Guildhall Essex Co. VT, Arthur Stanley Pease 35841 (KY);

WEST VIRGINIA. Berkeley County—519 Faulkner Ave., Federick Thompson (WVA); Grant County—Greenland Gap, J. A. Labriola (WVA); Greenbrier County—Camp Wood, Homer Duppstadt (WVA); Alvon, Allyne Shisler (WVA); Along Greenbrier River Trail 1 miles below Remick-Auto road, William N. Grafton (WVA); Anthony's Creek, Allyne Shisler (WVA); Hancock County—Lower Laurel Trail Wilderness Area, J. F. Clovis #1981 (WVA); Tomlinson Run Park, Russell West 533 (WVA); Tomlinson Run State Park, John S. Bonar 2-1 (WVA); Mercer County—Above camp creek road at sharp curve east of Brush Creek interchange, William N. Grafton (WVA); Banks of Bluestone River and Brush Creek, East of WV turnpike rest area., William N. Grafton (WVA); Brush Creek Falls, Meade McNeill (WVA); Mineral County—Sulfur to Hartmansville Rd along Emory's Run, Melvin Brown (WVA); Above the Potomac River., Melvin Brown (WVA); Pendleton County—Near Franklin, Elevation 1,700', Gerald Swank (WVA); , David B. Pingley 1683 (WVA); Near Franklin, Elevation 1,700', Gerald Swank (WVA); Pleasants County—Above Sugar Creek; 0.1 - 0.2 mi. SE., Co. Rt. 3012 & 0.3 mi, E., Co. Rt. 7 at Twiggs, Lafayette Distr., Allison W. Cussick 23,165 (WVA); Pocahontas County—Cranberry Glades, (WVA); Cranberry Glades, P. D. Strausbaugh 789 (WVA); Buckskin Res., J. B. Hinkle (WVA); Head of Greenbrier River, A. B. Brooks (WVA); Cranberry Glades, John L. Sheldon (WVA); Near Huntersville, F. W. Hunnewell 19,793 (WVA); William's River, A. D. Hopkins (WVA); Above upper Cranberry River & swamp, above Big Glade of Cranberry Glades, 3400 ft., G. B. Rossbach 73100 (WVA); Preston County—Cranesville, Mr. H. A. Davis 2698 (WVA); In cemetery - probably transplanted from swamp near where it occurs in small amounts, Cranesville, John L. Sheldon 1476 (WVA); Cranesville, W. E. Ramsey (WVA); Randolph County—Huttonsville, Rodney L. Bartgis (WVA); 4 miles above Spruce-Shavers Fork, Roger Findley 132 (WVA); Just below Fish Hatchery Run & sw. of Cheat Bridge, Alt. 3560 ft., George B. Rossbach 1313 (WVA); Blister Run, R. E. Henderson (WVA); Sinks of Gandy, James A. Stewart 856 (WVA); Summers County—0.75 mi E of Barger Spring, L. L. Gaddy (TENN); Big Stony Creek, Rodney L. Bartgis (WVA); Barger Springs, Weldon Boone 438 (WVA); Near mouth of Stony Creek/Barger Springs, William N. Grafton (WVA); Tucker County—5 m. S.E. of Davis, E. T. Wherry (WVA); Wetzel County—Along Fish Creek 1 to 3 mi. West of Littleton, Oscar Haught 397 (WVA); Wyoming County—Still Run, Cabin Creek, Dana Stike Evans (WVA); Mullens, Dana Stike Evans (WVA); Mullens, Dana Stike Evans (WVA);

4. *Taxus cuspidata* Siebold & Zuccarini

U.S.A.

CALIFORNIA. Los Angeles County—Claremont, Don A. Emerson (WVA);

KENTUCKY. Bullitt County—Contact Station area., Charles R. Gunn 1290 (KY); Fayette County—Rose St. Lexington KY, Richard Knodel (KY); Lexington cemetery cultivated, Phill Fisher (KY); UK Campus, Claude F. Wade 6 (KY); Courtney Ave backyard, Robert R. Pace SOTINKY36 (EKY); Jefferson County—Left end of row of evergreens flanking Central Ave. entrance., Ruth B. (Alford) MacFarlane 4313 (No. 29) (KY); Madison County — The Ravine on EKU's campus, Robert R. Pace SOTINKY55 (EKY);

NEW JERSEY. New Brunswick County—Rutgers University Campus, Daniel R. Mock (WVA);

OHIO. Wayne County—Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A30-125 (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A30-182 (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A30-257 (KNK); Shelby County—Memphis. Cultivated - 486 St. Nick Drive. Rehd. Ed. 2-3., Elizabeth M. Browne 72D20.6 (TENN);

5. ***Taxus floridana*** Nuttall ex Chapman

U.S.A.:

FLORIDA. Alachua County—Gainesville University of Florida campus, Cultivated south side of Hume Auditorium, Bian Tan 172 (WVA); Calhoun County—Appalachicola River, near Bristol., W. C. Muenscher 14309 (WVA); Liberty County—Torreya State Park Ca 10 miles N of Bristol, John W. Thieret (KNK); Torreya State Park; near stone bridge., B. Eugene Wofford 47221 (TENN); , John K. Small (WVA); Rock Bluff Florida, F. S. Blanton 7050 (WVA); Branch of Big Sweetwater Creek, E side of State Rd. 270. N of Big Sweetwater Creek. Between Torreya State Park and State Rd. 12, Steven R. Hill 19143 (WVA);

6. ***Taxus globosa*** Schlectendahl

Mexico:

N/A. Tamaulipas County—Rancho del Cielo to Ojo de Augua del Indio below 5000 ft. Above Gomez Farias - Tamaulipas; Mexico, A. J. Sharp 50/50178 (TENN);

7. ***Taxus mairei*** (Lemée & Leviellé) Shiu-Ying Hu ex Liu

China:

Jiangsu. Nanjing County—Cultivated in the Nanjing Botanical Garden Memorial Sun Yat-Sen of the Jiangsu Institute of Botany, Sino-American Purple Shan Botanical Expedition (SAPSBE); T. R. Dudley 45324 (TENN);

8. ***Taxus × hunnewelliana*** **Rehder**

U.S.A.:

OHIO. Wayne County—Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A31-197 (KNK);

9. ***Taxus × media*** **Rehder**

U.S.A.:

OHIO. Wayne County—Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A30-131 (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A31-84 (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret A31-123 (KNK); Living Herbarium of Taxus at the Secrest Arboretum - Ohio Agricultural Research and Development Center - Wooster, John W. Thieret (KNK);

KENTUCKY. Madison County — East side of 3rd street near intersection of Woodland Ave., Robert R. Pace SOTINKY2 (EKY); East side of 3rd street between Main st. and Irvine Road, Robert R. Pace SOTINKY3 (EKY); Northeast corner of Jones building on EKU's campus, Robert R. Pace SOTINKY4 (EKY); In between Coates and Jones building on EKU's campus, Robert R. Pace SOTINKY5 (EKY); In between Coates and Jones building on EKU's campus, Robert R. Pace SOTINKY6 (EKY); Near Library on EKU's campus, Robert R. Pace SOTINKY7 (EKY); Corner of Moore building on EKU's campus, Robert R. Pace SOTINKY8 (EKY); Hedge to the left of the front of Roark building on EKU's campus, Robert R. Pace SOTINKY41 (EKY); Pyrimidal shaped to the left of front of Roark building on EKU's campus, Robert R. Pace SOTINKY43 (EKY); Wolfe County — Cottage Rd., Robert R. Pace SOTINKY33 (EKY); South side of hemlock lodge, Robert R. Pace SOTINKY34 (EKY); North side of hemlock lodge, Robert R. Pace SOTINKY35 (EKY); Menifee County — Cliff along HW 715, Robert R. Pace SOTINKY37 (EKY);

APPENDIX B: Scanning Electron Micrographs

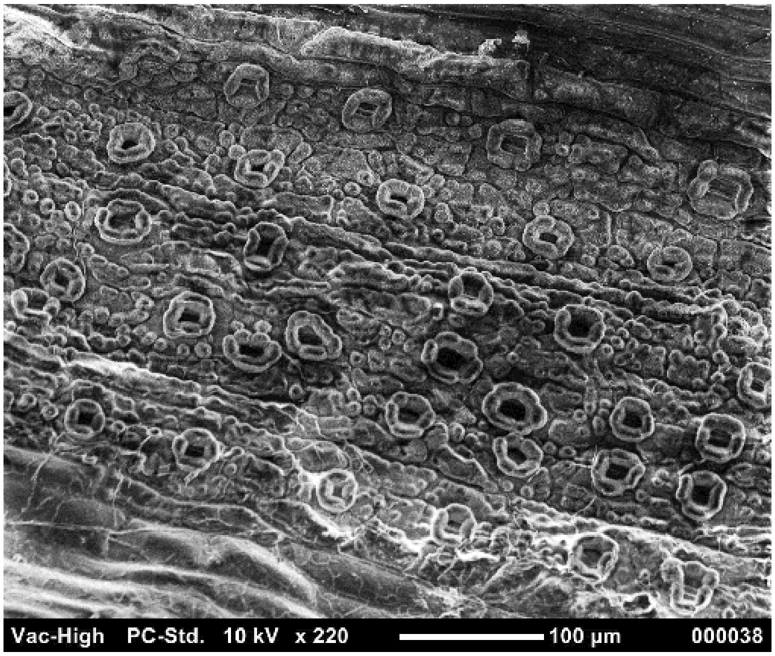


Figure 6. SEM of *Taxus canadensis* abaxial leaf surface showing 5 stomates per band with interspersed papillose cells.

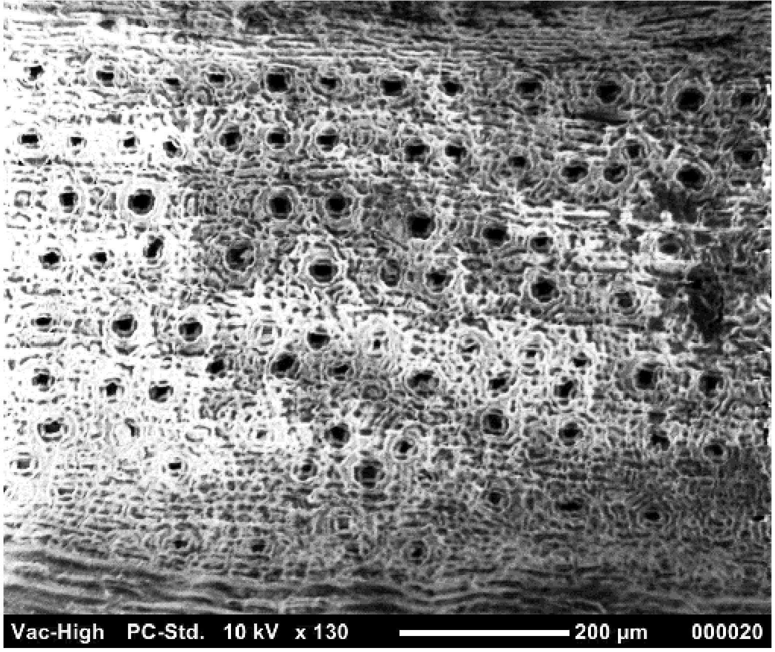


Figure 7. SEM of *Taxus baccata* abaxial leaf surface showing 10 stomates per band with interspersed papillose cells.

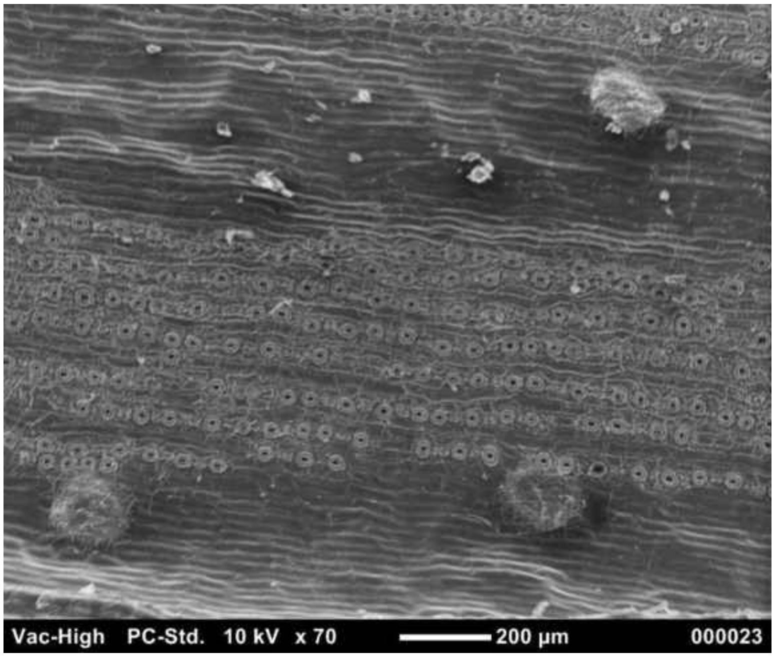


Figure 8. SEM of *Taxus* × *hunnewelliana* abaxial leaf surface showing 9 irregular stomates per band with interspersed papillose cells confined to between stoma.

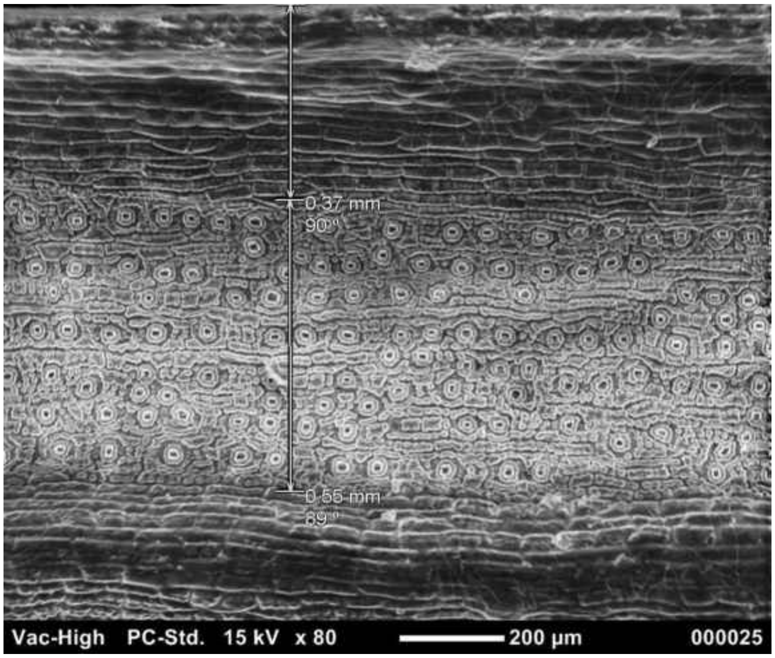


Figure 9. SEM of *Taxus floridana* abaxial leaf surface showing 9 stomates per narrow band with interspersed papillose cells.

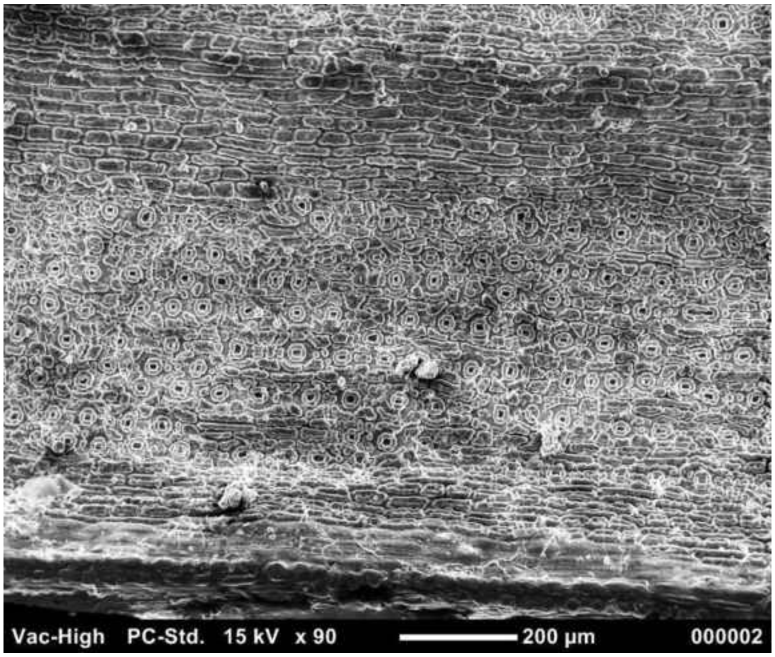


Figure 10. SEM of *Taxus baccata* ‘compacta’ abaxial leaf surface showing 9 stomates per irregular band with interspersed papillose cells.

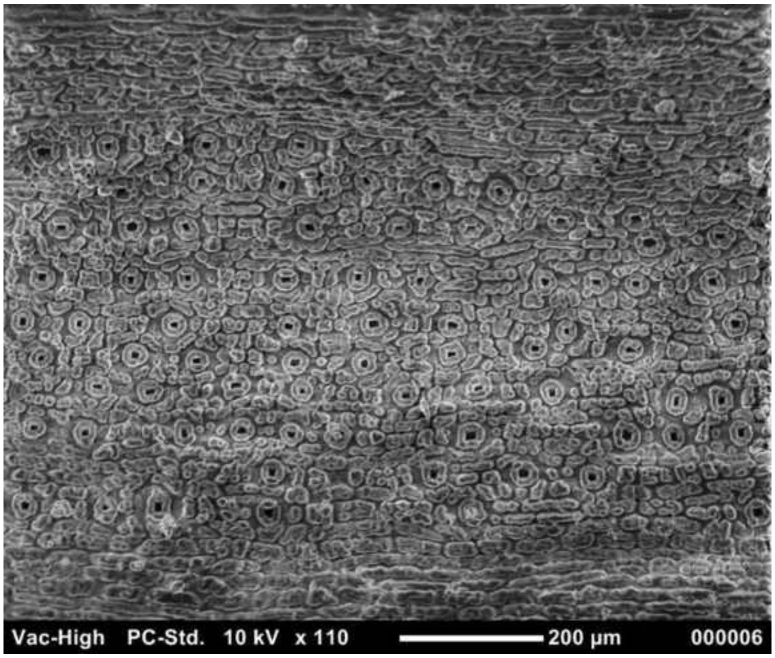


Figure 11. SEM of *Taxus baccata* ‘nigra’ abaxial leaf surface showing 9 stomates per narrow band with densely papillose cells.

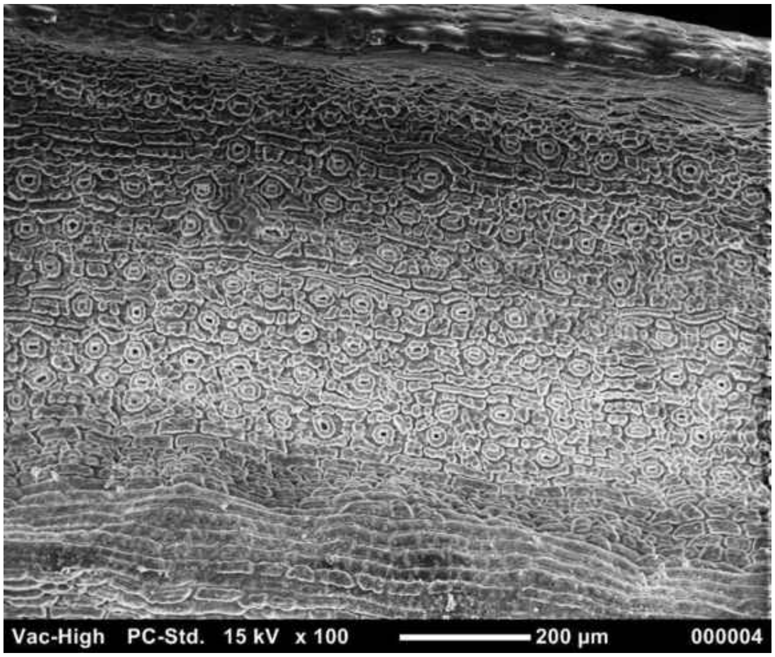


Figure 12. SEM of *Taxus baccata* ‘repandens’ abaxial leaf surface showing 9 stomates per irregular band with interspersed papillose cells.

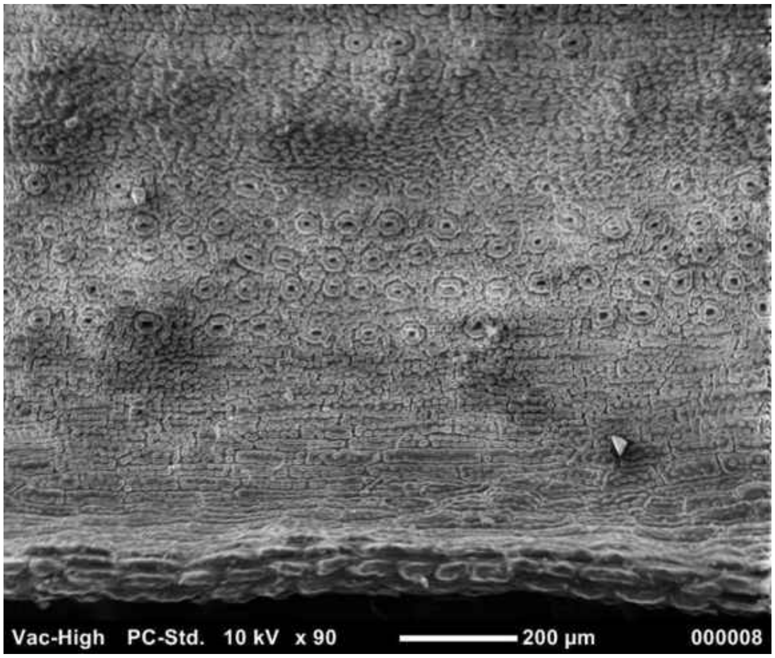


Figure 13. SEM of *Taxus brevifolia* abaxial leaf surface showing 5 stomates per band with interspersed papillose cells.

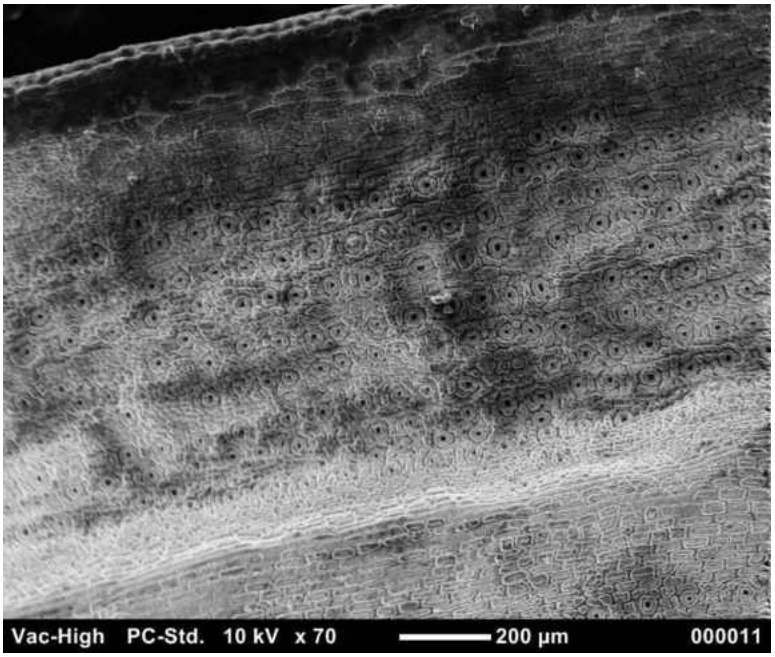


Figure 14. SEM of *Taxus cuspidata* ‘robusta’ abaxial leaf surface showing 10 stomates per broad band with interspersed papillose cells.

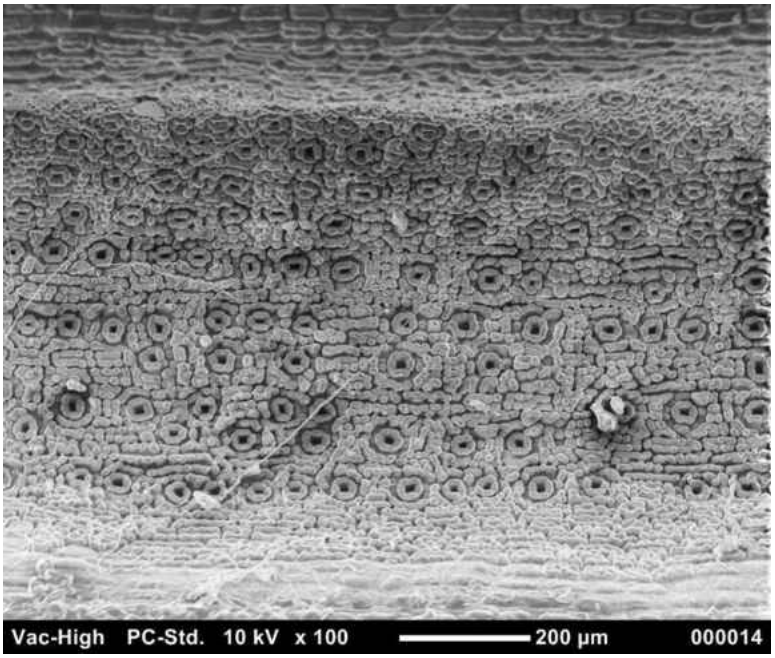


Figure 15. SEM of *Taxus cuspidata* abaxial leaf surface showing 10 stomates per broad band with densely papillose cells.

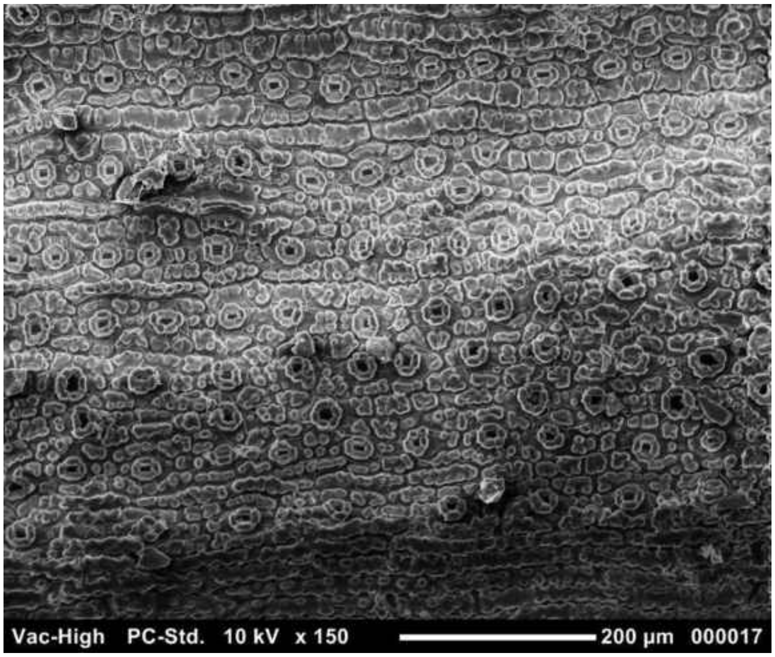


Figure 16. SEM of *Taxus globosa* abaxial leaf surface showing 8 stomates per broad band with interspersed papillose cells.

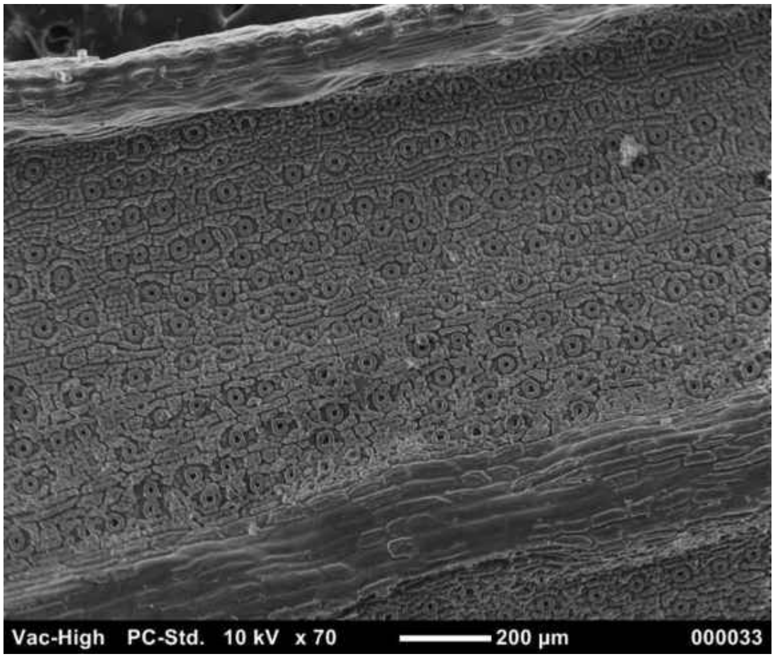


Figure 17. SEM of *Taxus* × *media* abaxial leaf surface showing 11+ stomates per wide band with interspersed papillose cells.

APPENDIX C: Taxanes

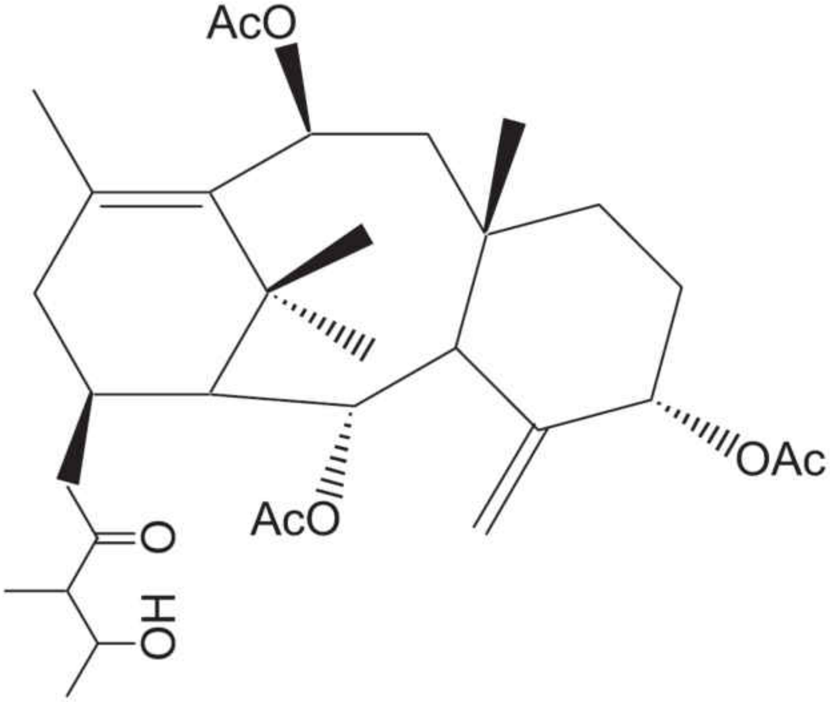


Figure 18. Yunnanxane. 2α,5α,10β-triacetoxytaxa-4(20),11-dien-14-yl 3-hydroxy-2-methylbutanoate. $C_{31}H_{46}O_9$ 562.69 g·mol^{-1}. $[M + NH_4]^+$ with expected *m/z* of 580.35.

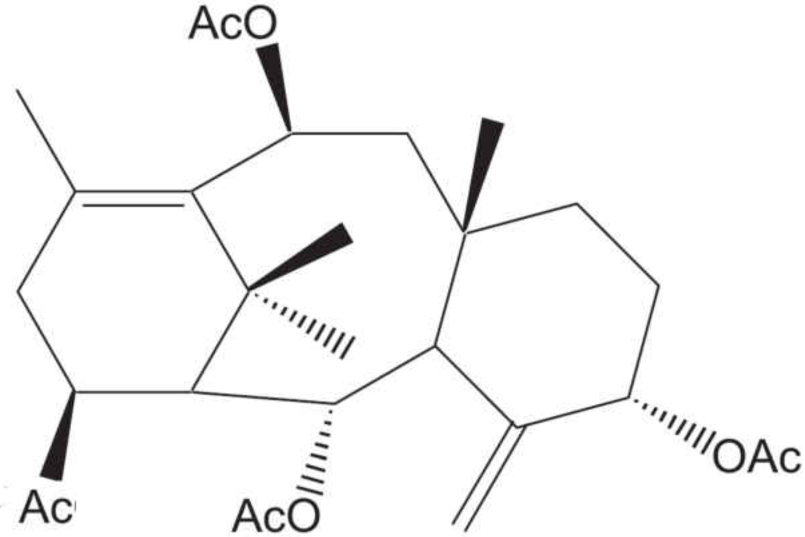


Figure 19. 2α,5α,10β,14β-tetraacetoxy-4(20),11-taxadiene. $C_{28}H_{40}O_8$ 504.61 g·mol^{-1}. $[M + NH_4]^+$ with expected *m/z* of 522.31.

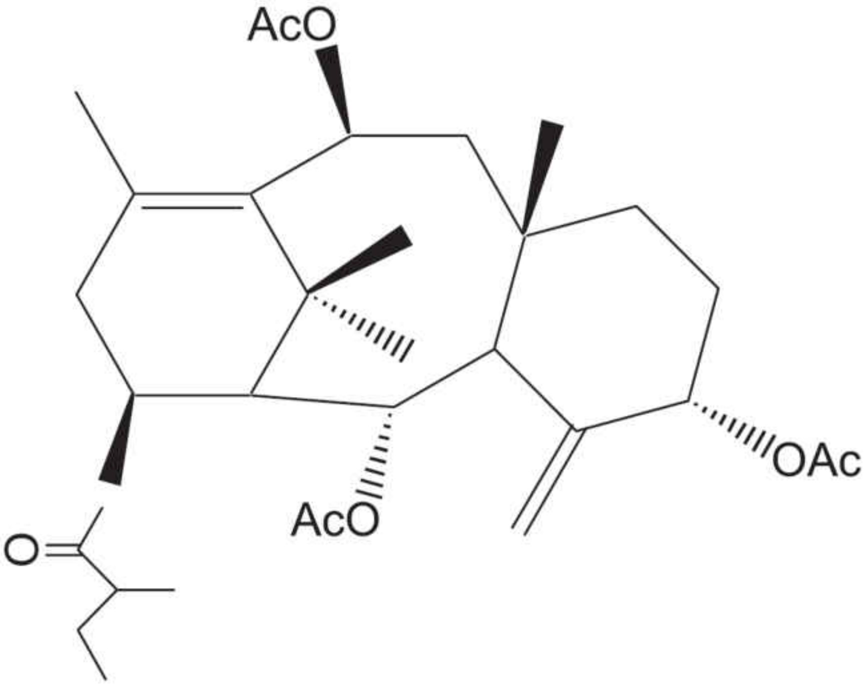


Figure 20. 2α,5α,10β-triacetoxy-14β-(2-methyl)-butyryloxy-4(20),11-taxadiene. $C_{31}H_{46}O_8$ 546.69 g·mol^{-1}. $[M + NH_4]^+$ with expected *m/z* of 564.36.

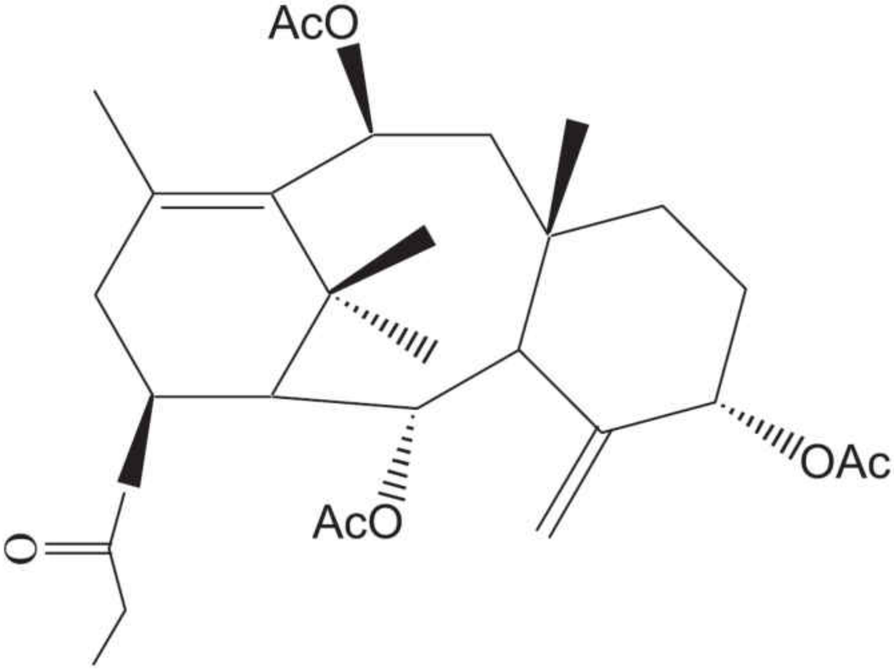


Figure 21. 2α,5α,10β-triacetyoxy-14β-propionyloxy-4(20),11-taxadiene. $C_{29}H_{42}O_8$ 518.61 g·mol^{-1}. $[M + NH_4]^+$ with expected *m/z* of 536.32.

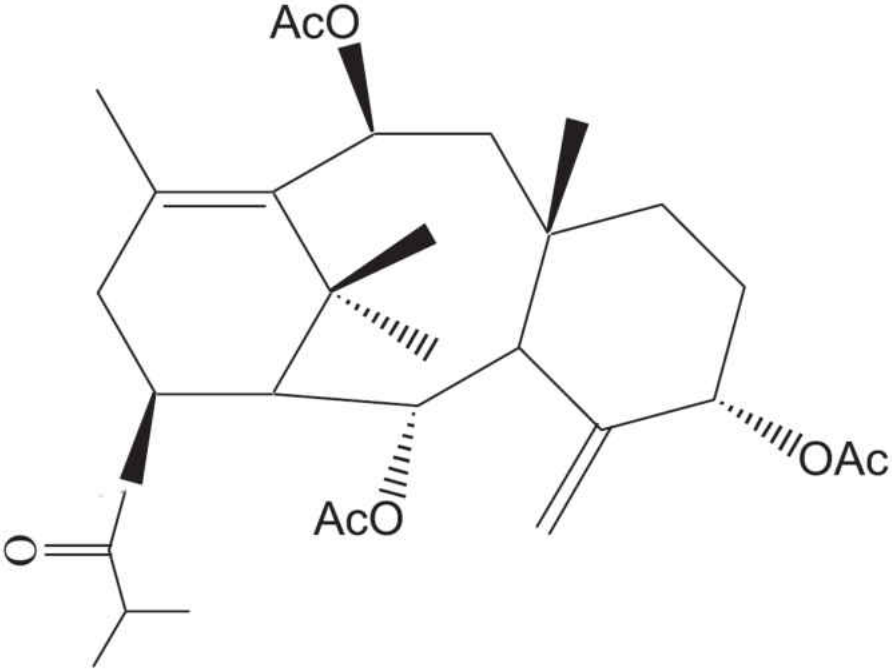


Figure 22. 2α,5α,10β-triacetyoxy-14β-isobutyryloxy-4(20),11-taxadiene. $C_{30}H_{44}O_8$ 532.66 g·mol^{-1}. $[M + NH_4]^+$ with expected *m/z* of 550.34.

APPENDIX D: Mass Spectra

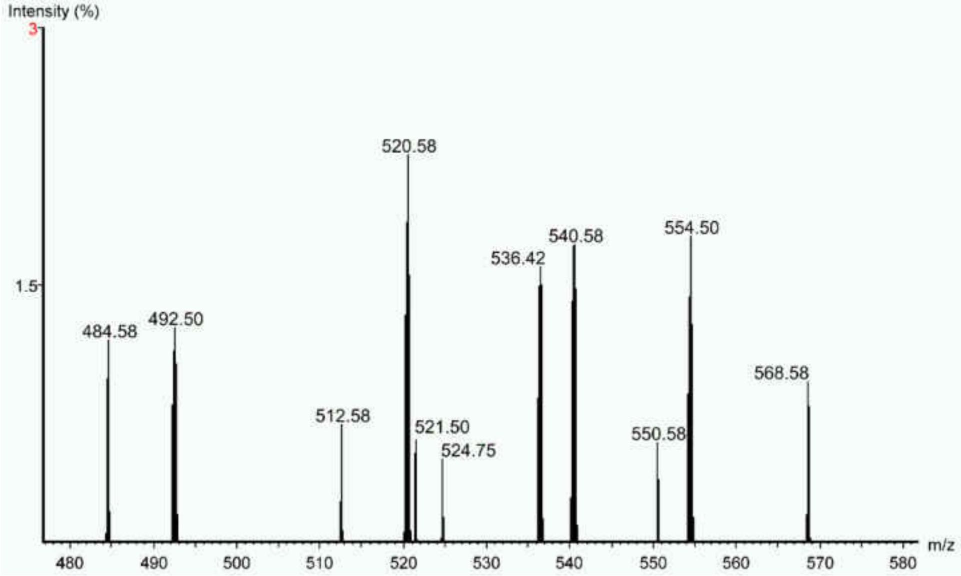


Figure 23. Mass spectrum of leaves of *Taxus baccata* (SOTINKY01) showing taxanes at *m/z* 536 $[M + NH_4]^+$, and *m/z* 550 $[M + NH_4]^+$.

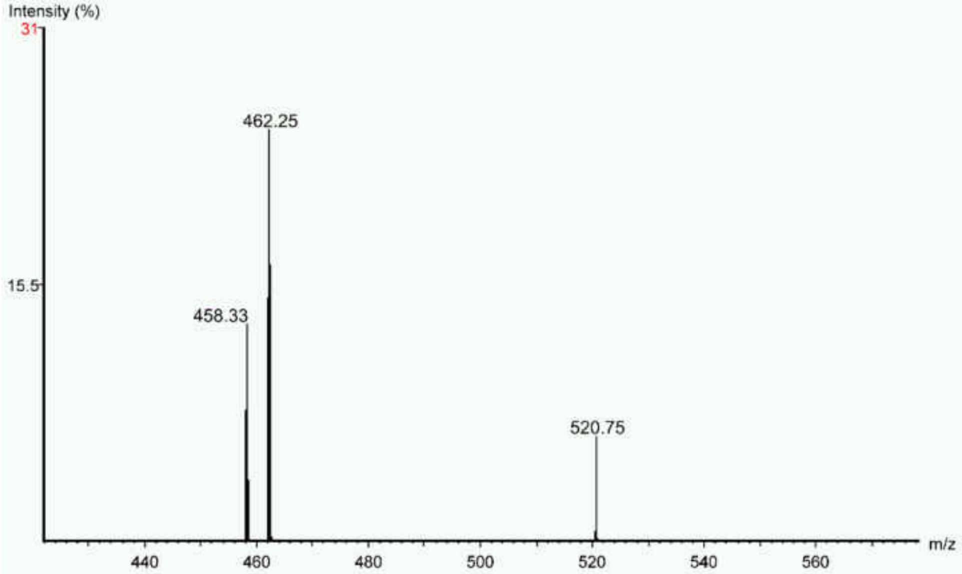


Figure 24. Mass spectrum of stem of *Taxus baccata* (SOTINKY01) showing none of the five taxanes present.

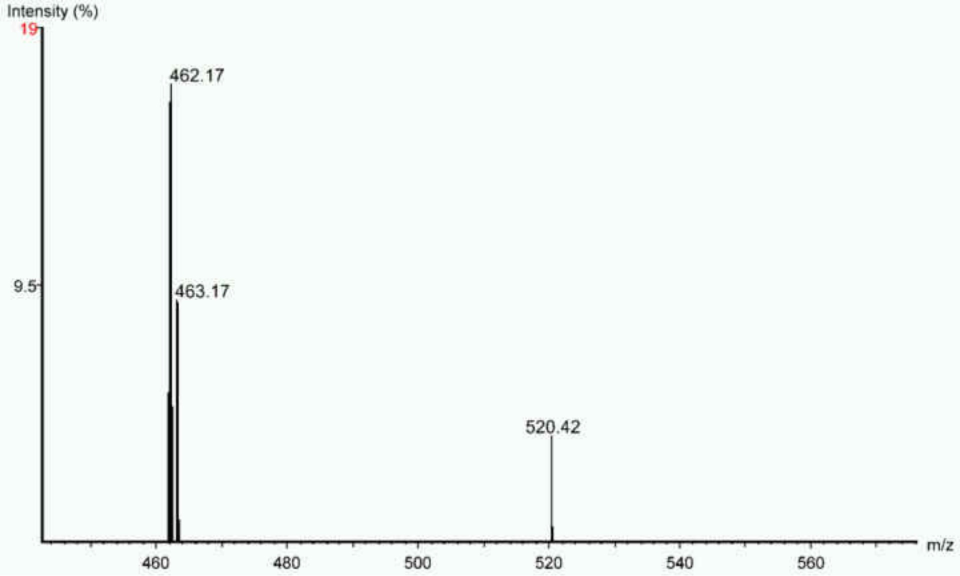


Figure 25. Mass Spectrum of leaves of *Taxus* × *media* (SOTINKY02) showing none of the five taxanes present.

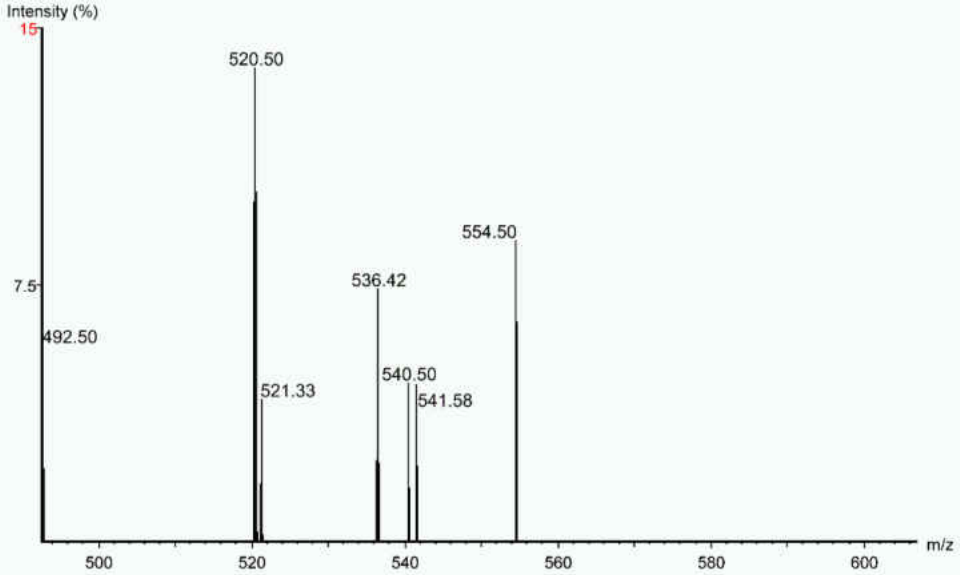


Figure 26. Mass Spectrum of stem of *Taxus* × *media* (SOTINKY02) showing the presence of a taxane at *m/z* 536 $[M + NH_4]^+$.

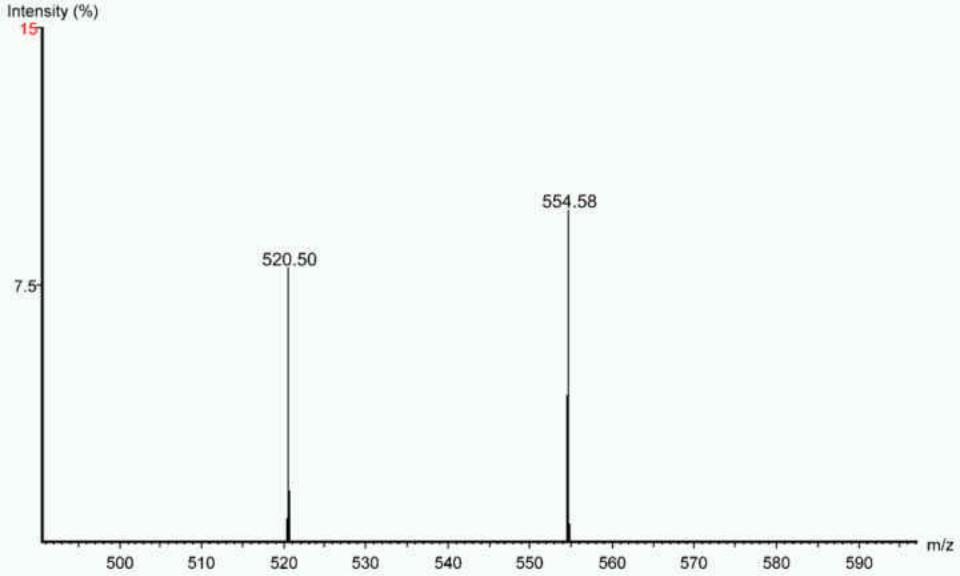


Figure 27. Mass Spectrum of leaves of *Taxus* × *media* (SOTINKY03) showing none of the five taxanes present.

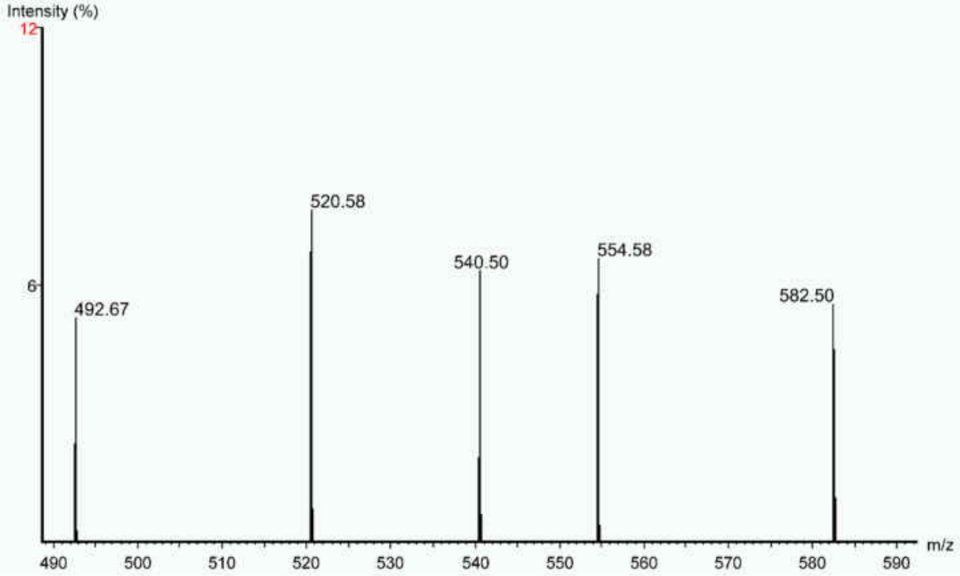


Figure 28. Mass Spectrum of stem of *Taxus* × *media* (SOTINKY03) showing none of the five taxanes present.

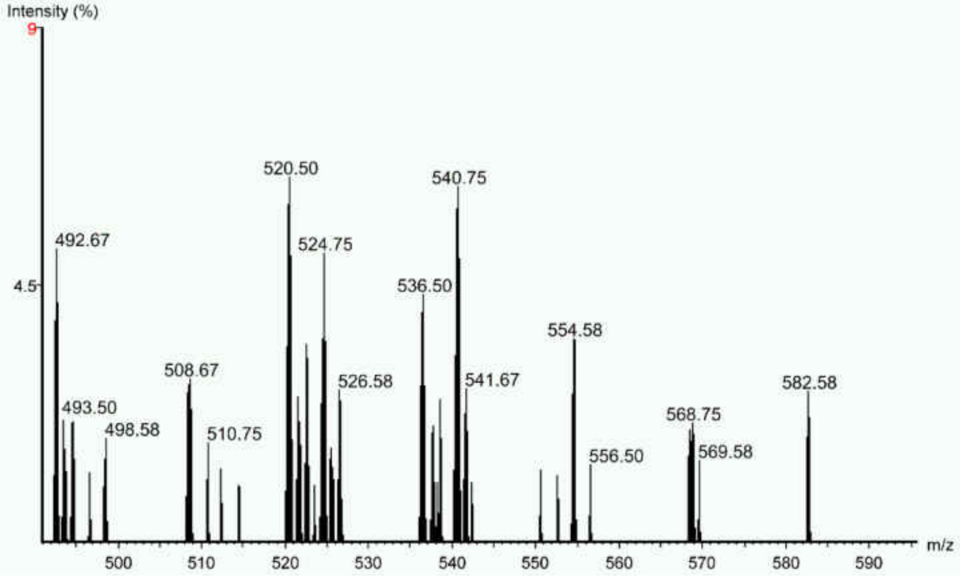


Figure 29. Mass Spectrum of leaves of *Taxus* × *media* (SOTINKY07), showing the presence of a taxane at *m/z* 536 $[M + NH_4]^+$.

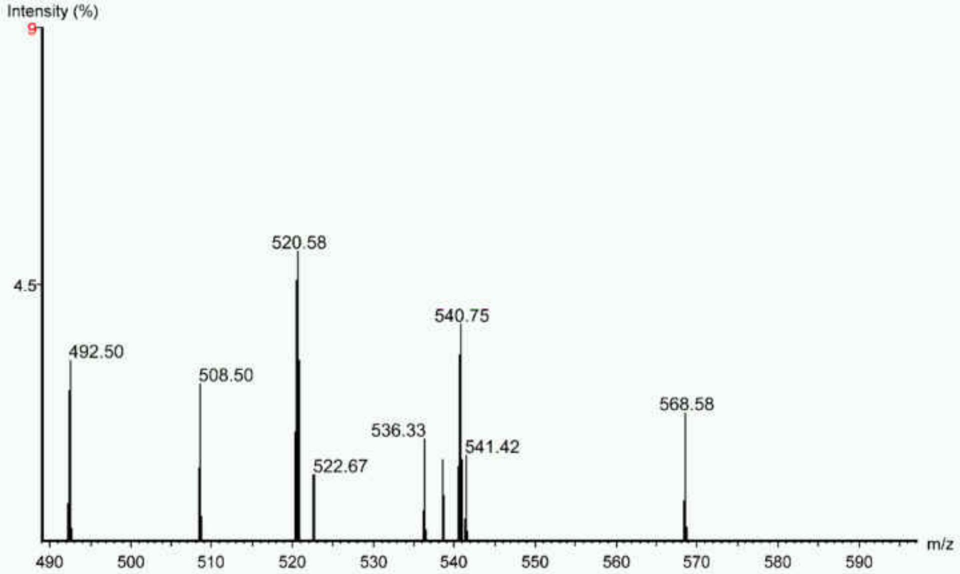


Figure 30. Mass Spectrum of the stem of *Taxus* × *media* (SOTINKY07) showing taxanes at *m/z* 522 $[M + NH_4]^+$, and *m/z* 536 $[M + NH_4]^+$.

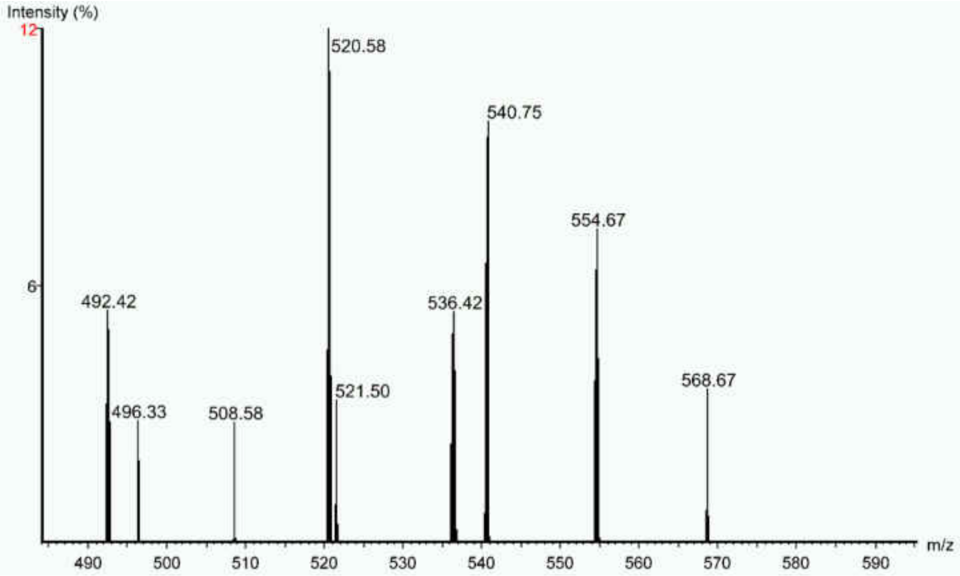


Figure 31. Mass Spectrum of the leaves of *Taxus* × *media* (SOTINKY08) showing a taxane at *m/z* 536 $[M + NH_4]^+$.

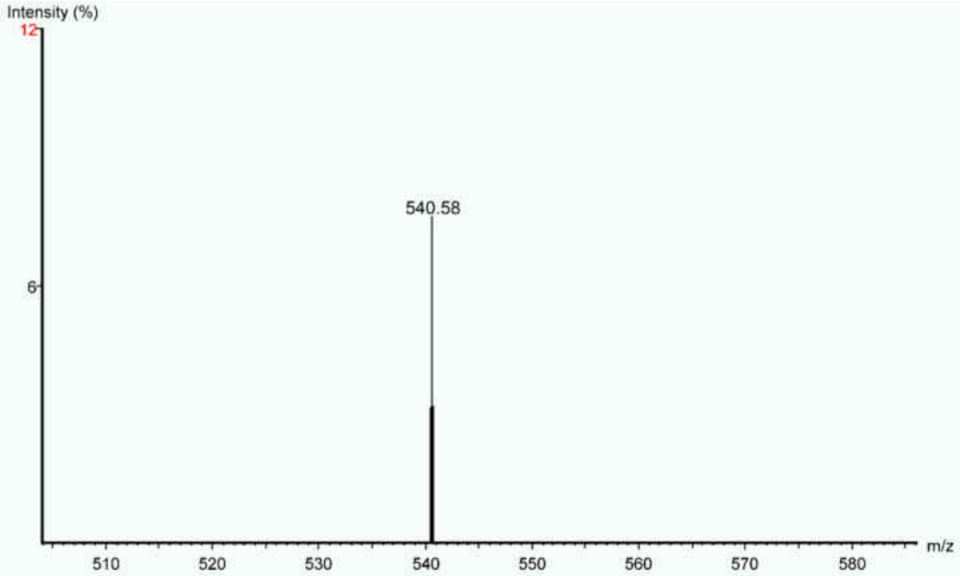


Figure 32. Mass Spectrum of the stem of *Taxus* × *media* (SOTINKY08) showing none of the five taxanes present.

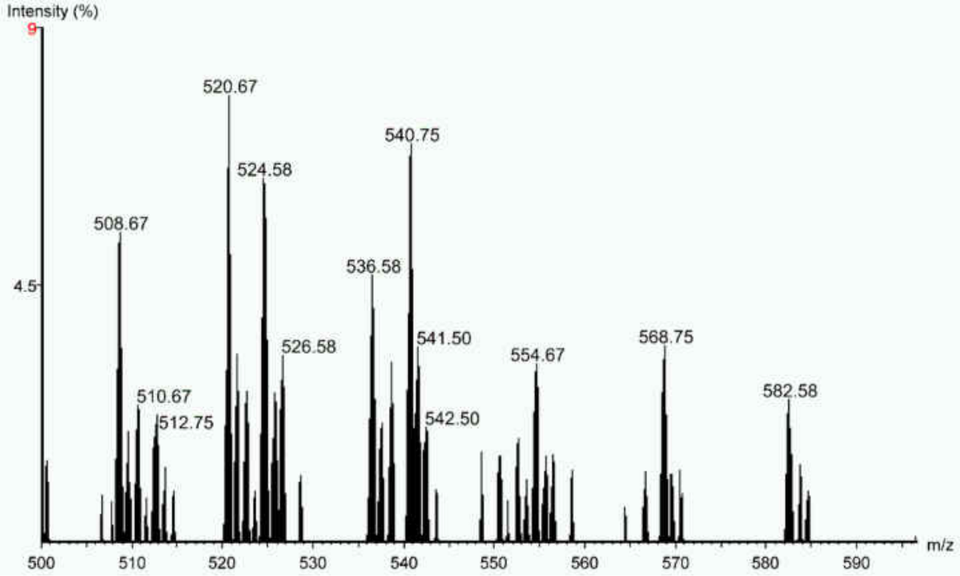


Figure 33. Mass Spectrum of the leaves of *Taxus canadensis* (SOTINKY33), showing the presence of taxanes at *m/z* 536 $[M + NH_4]^+$, *m/z* 550 $[M + NH_4]^+$, and *m/z* 564 $[M + NH_4]^+$.

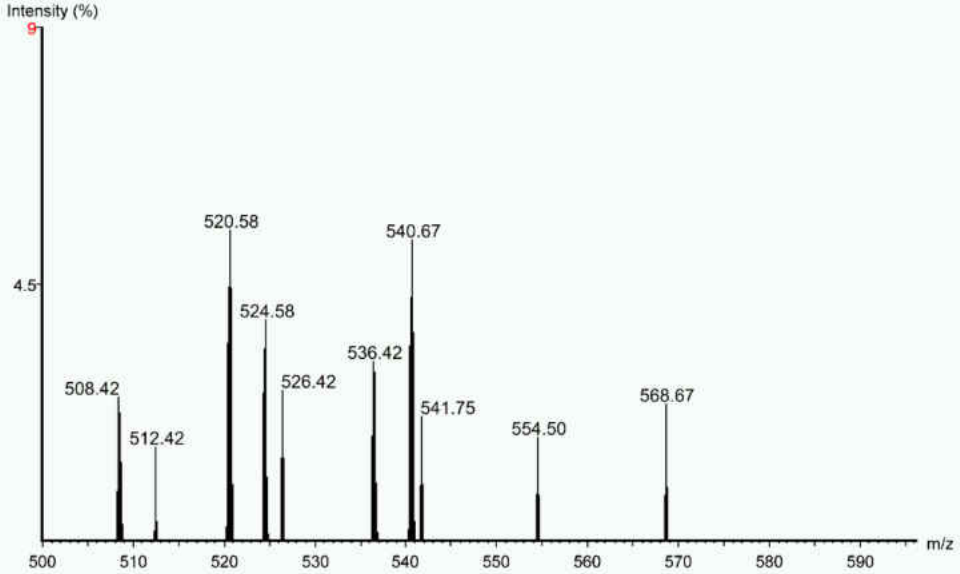


Figure 34. Mass Spectrum of the stem of *Taxus canadensis* (SOTINKY33), showing a taxane at *m/z* 536 $[M + NH_4]^+$.

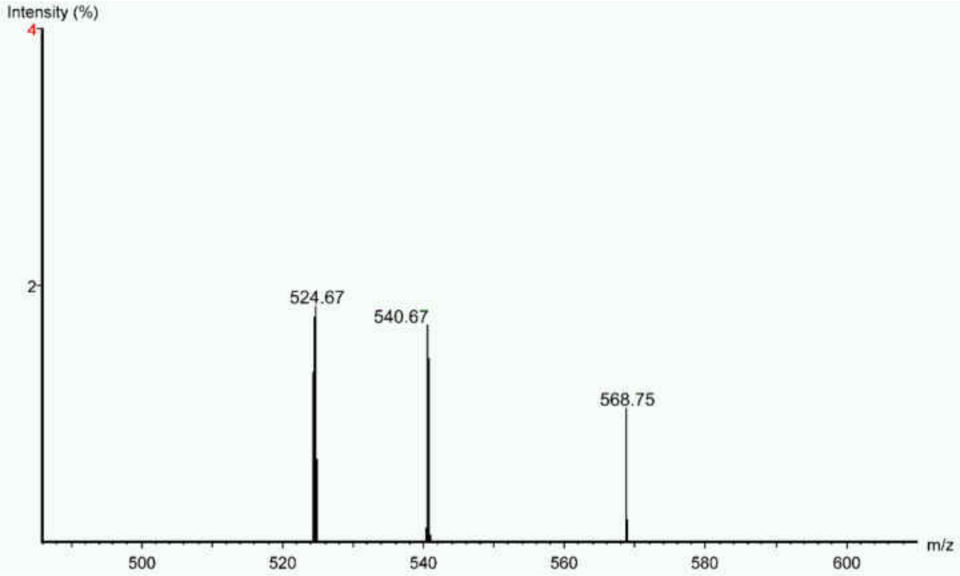


Figure 35. Mass Spectrum of the leaves of *Taxus* × *media* (SOTINKY36) showing none of the five taxanes present.

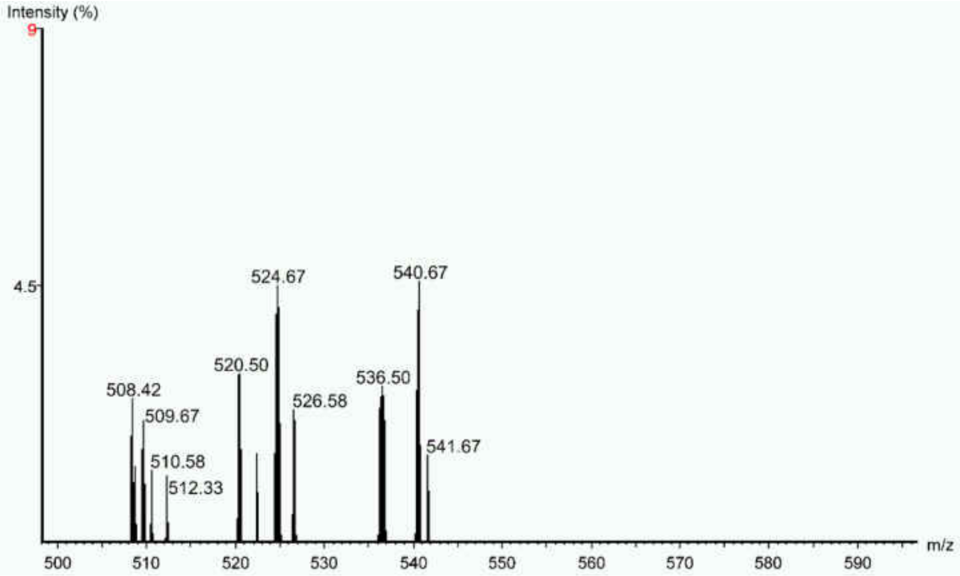


Figure 36. Mass Spectrum of the stem of *Taxus* × *media* (SOTINKY36), showing a taxane present at *m/z* 536 $[M + NH_4]^+$.

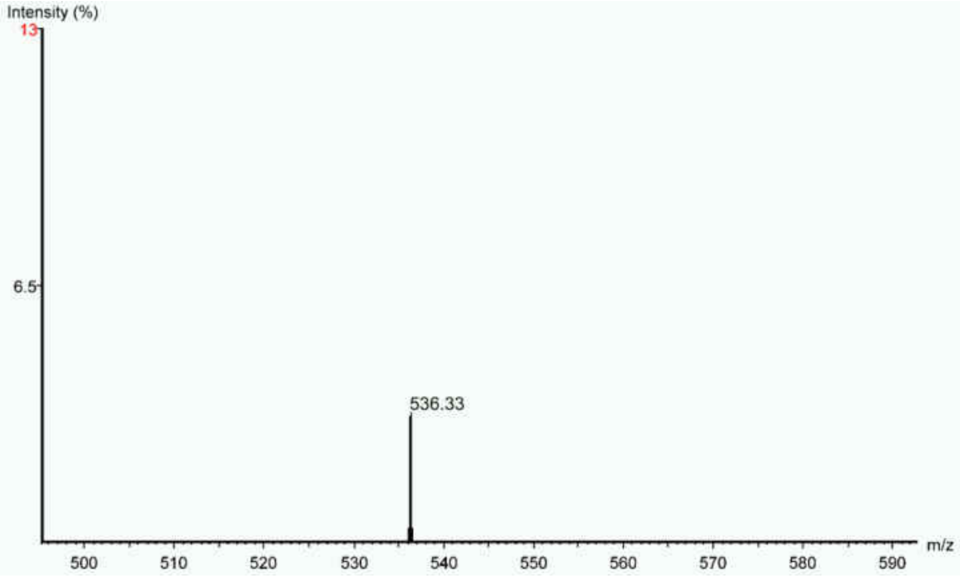


Figure 37. Mass Spectrum of the leaves of *Taxus* × *media* (SOTINKY42) showing the presence of a taxane at *m/z* 536 $[M + NH_4]^+$.

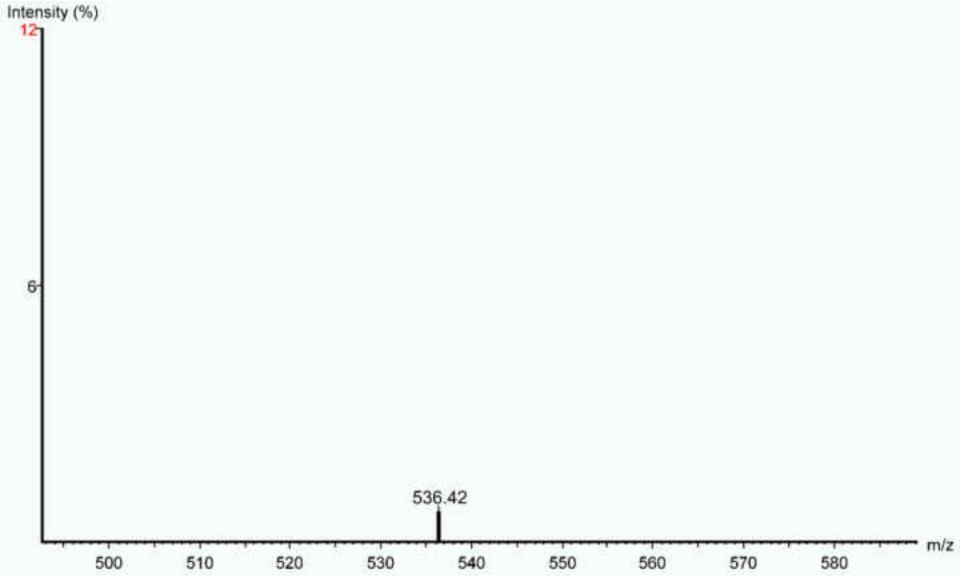


Figure 38. Mass Spectrum of the stem of *Taxus* × *media* (SOTINKY42), showing the presence of a taxanes at *m/z* 536 $[M + NH_4]^+$.

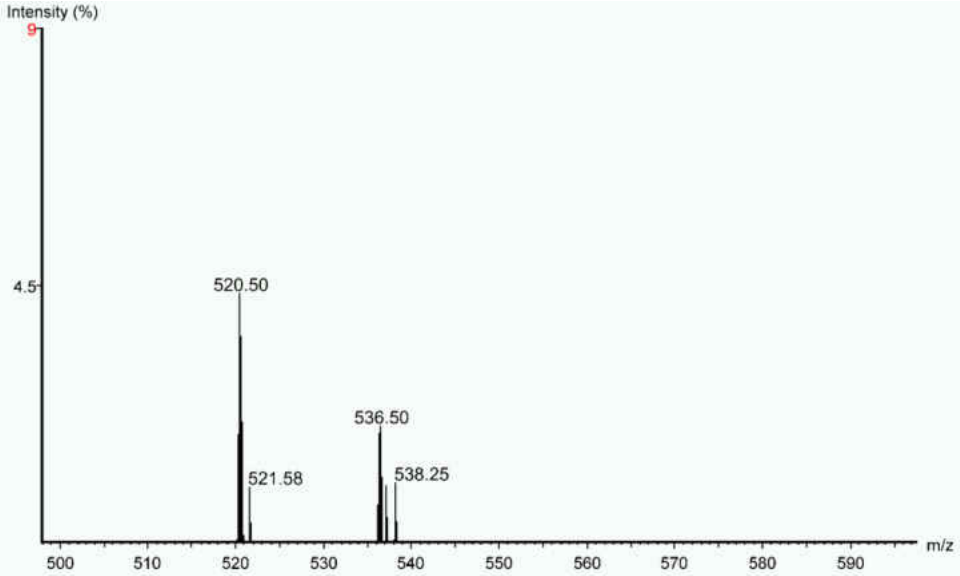


Figure 39. Mass Spectrum of the leaves of *Taxus cuspidata* (SOTINKY53), showing the presence of a taxane at *m/z* 536 $[M + NH_4]^+$.

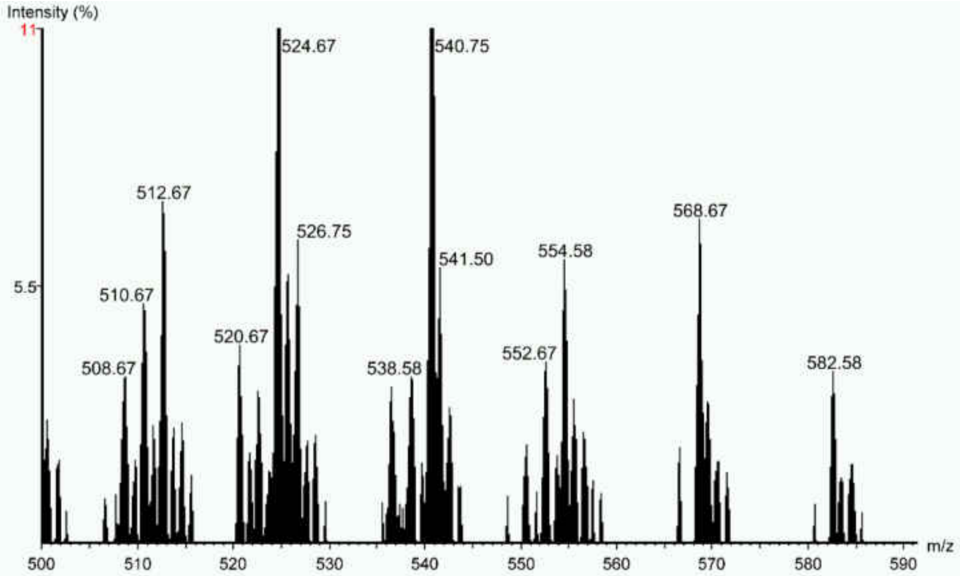


Figure 40. Mass Spectrum of the stem of *Taxus cuspidata* (SOTINKY53), showing taxanes at *m/z* 522 $[M + NH_4]^+$, *m/z* 536 $[M + NH_4]^+$, *m/z* 550 $[M + NH_4]^+$, and *m/z* 580 $[M + NH_4]^+$.

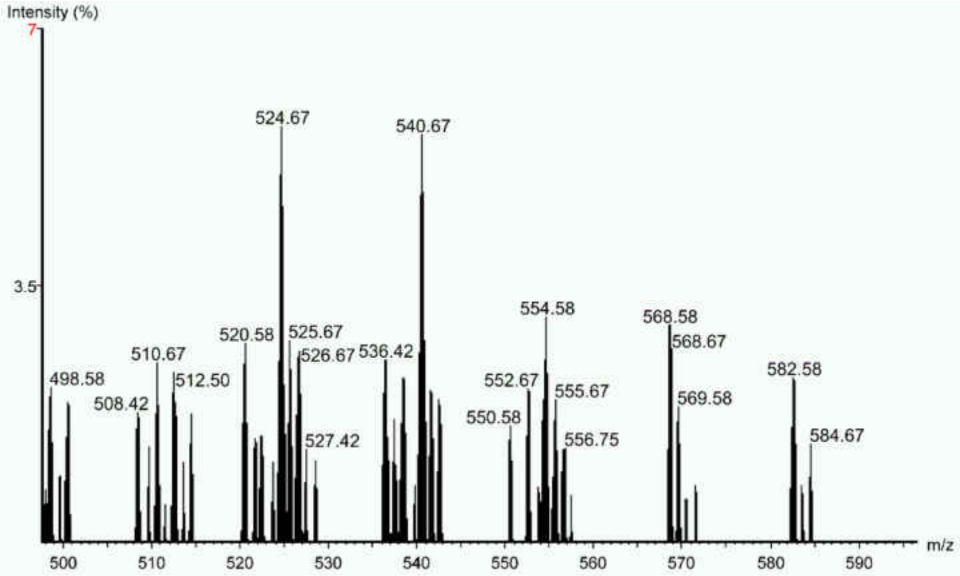


Figure 41. Mass Spectrum of the leaves of *Taxus cuspidata* (SOTINKY55) showing the presence of taxanes at *m/z* 536 $[M + NH_4]^+$, and *m/z* 550 $[M + NH_4]^+$.

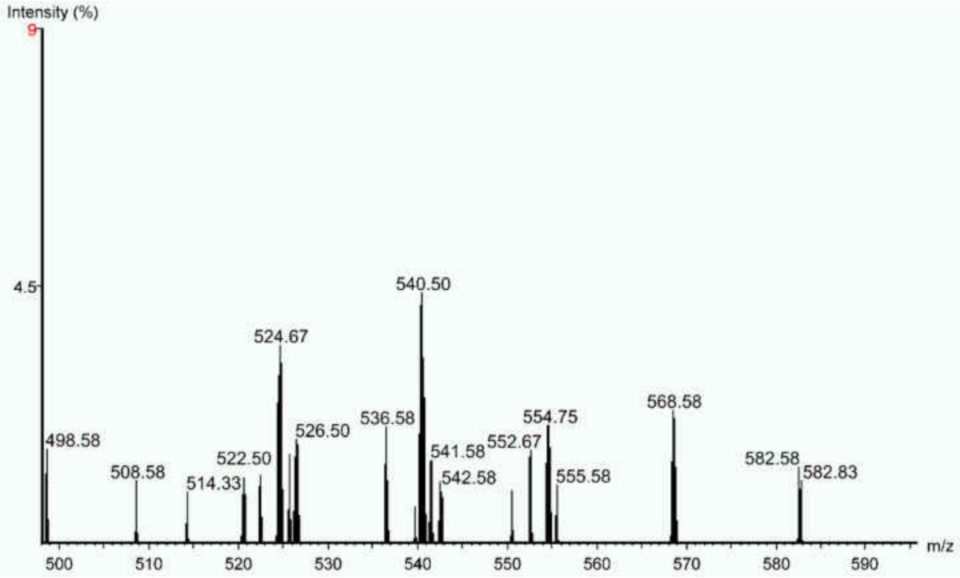
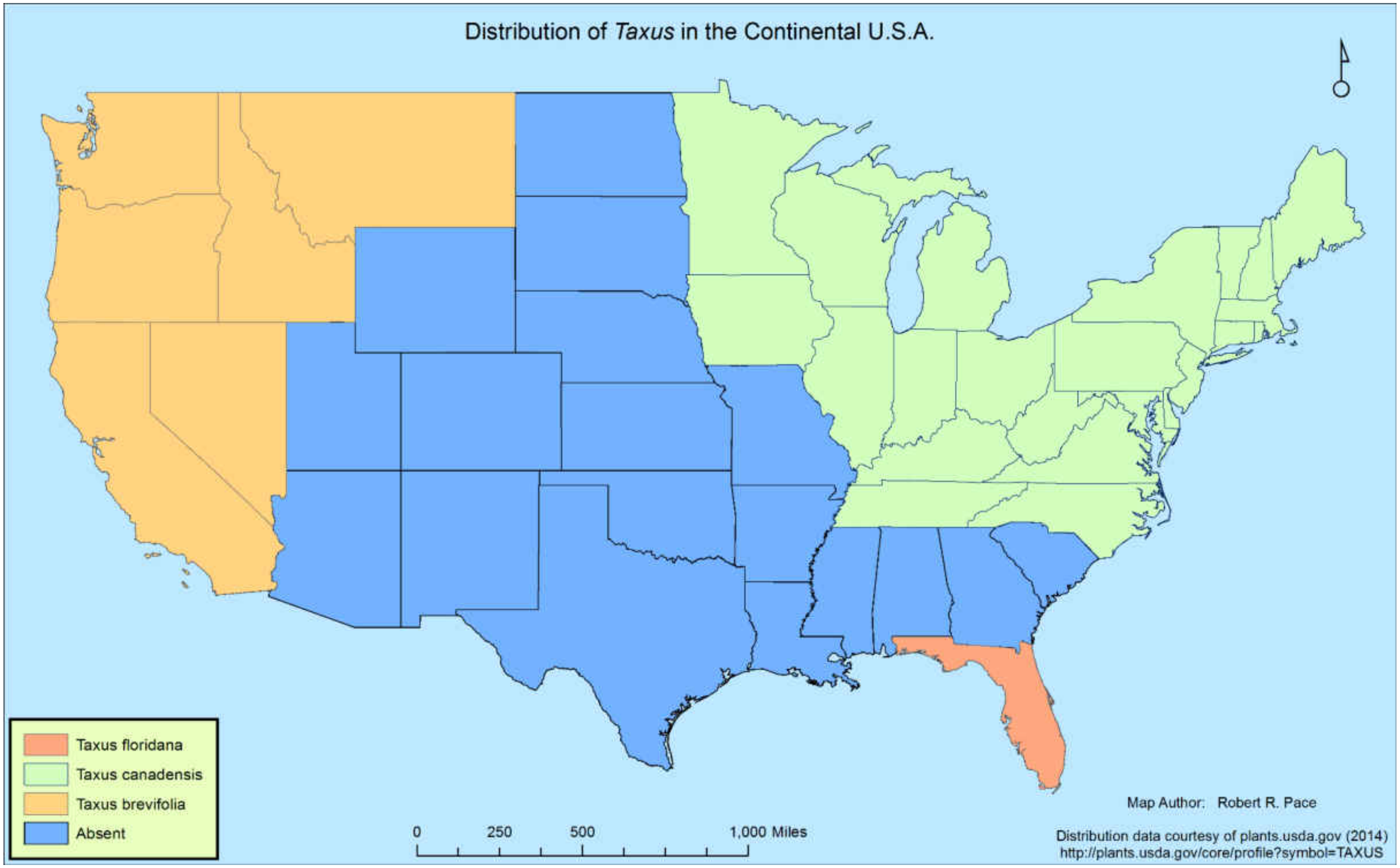


Figure 42. Mass Spectrum of the stem of *Taxus cuspidata* (SOTINKY55) showing the presence of taxanes at *m/z* 522 $[M + NH_4]^+$, *m/z* 536 $[M + NH_4]^+$, and *m/z* 550 $[M + NH_4]^+$.

APPENDIX E: Maps of Distribution in United States, Kentucky, & Nearby States



Map Author: Robert R. Pace

Distribution data courtesy of plants.usda.gov (2014)
http://plants.usda.gov/core/profile?symbol=TAXUS

Figure 43. Map of the continental U.S.A. showing distribution of *Taxus* species.

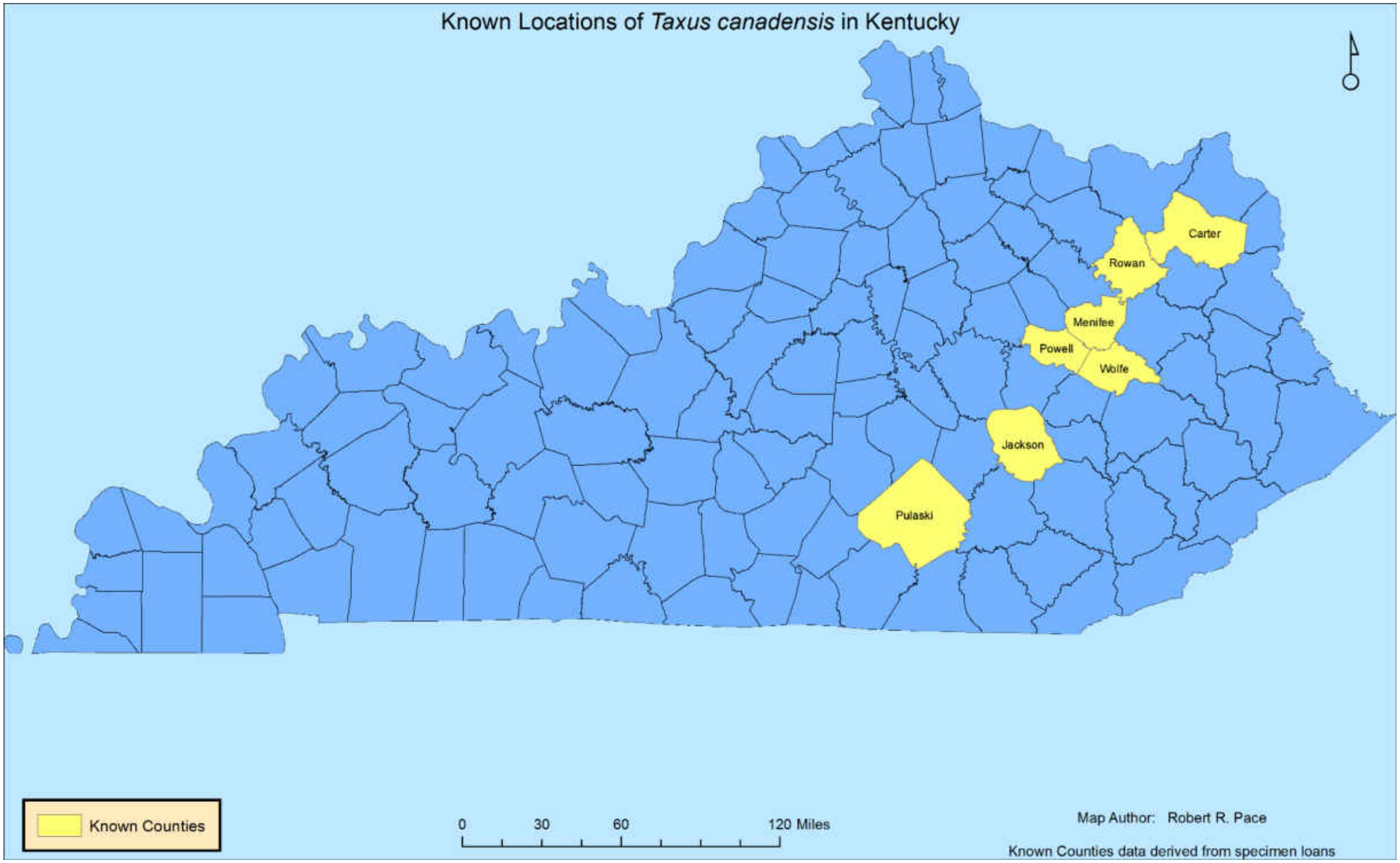


Figure 44. Map of Kentucky showing distribution of *Taxus canadensis*.

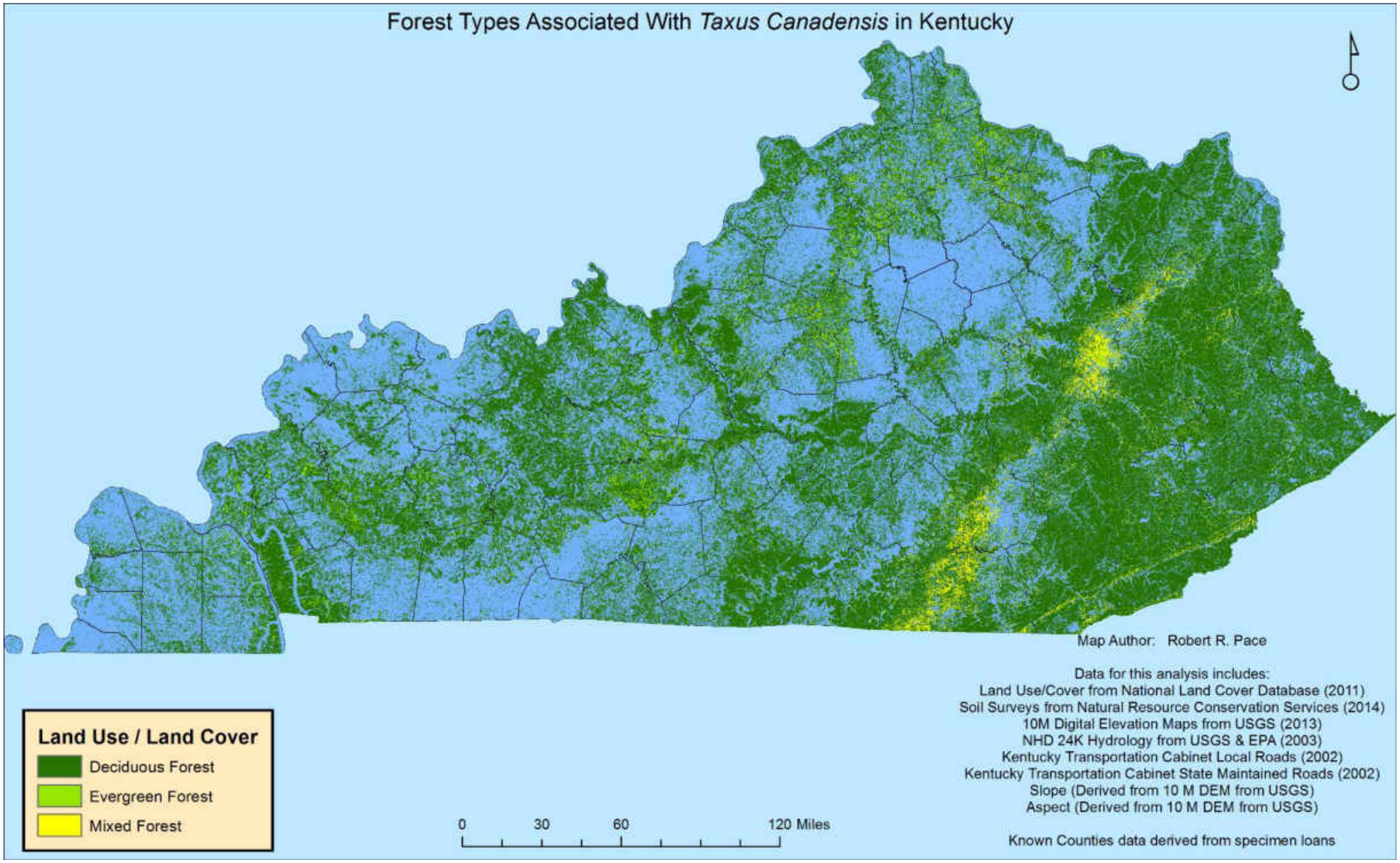


Figure 45. Map of Kentucky showing forest types associated with *Taxus canadensis*.

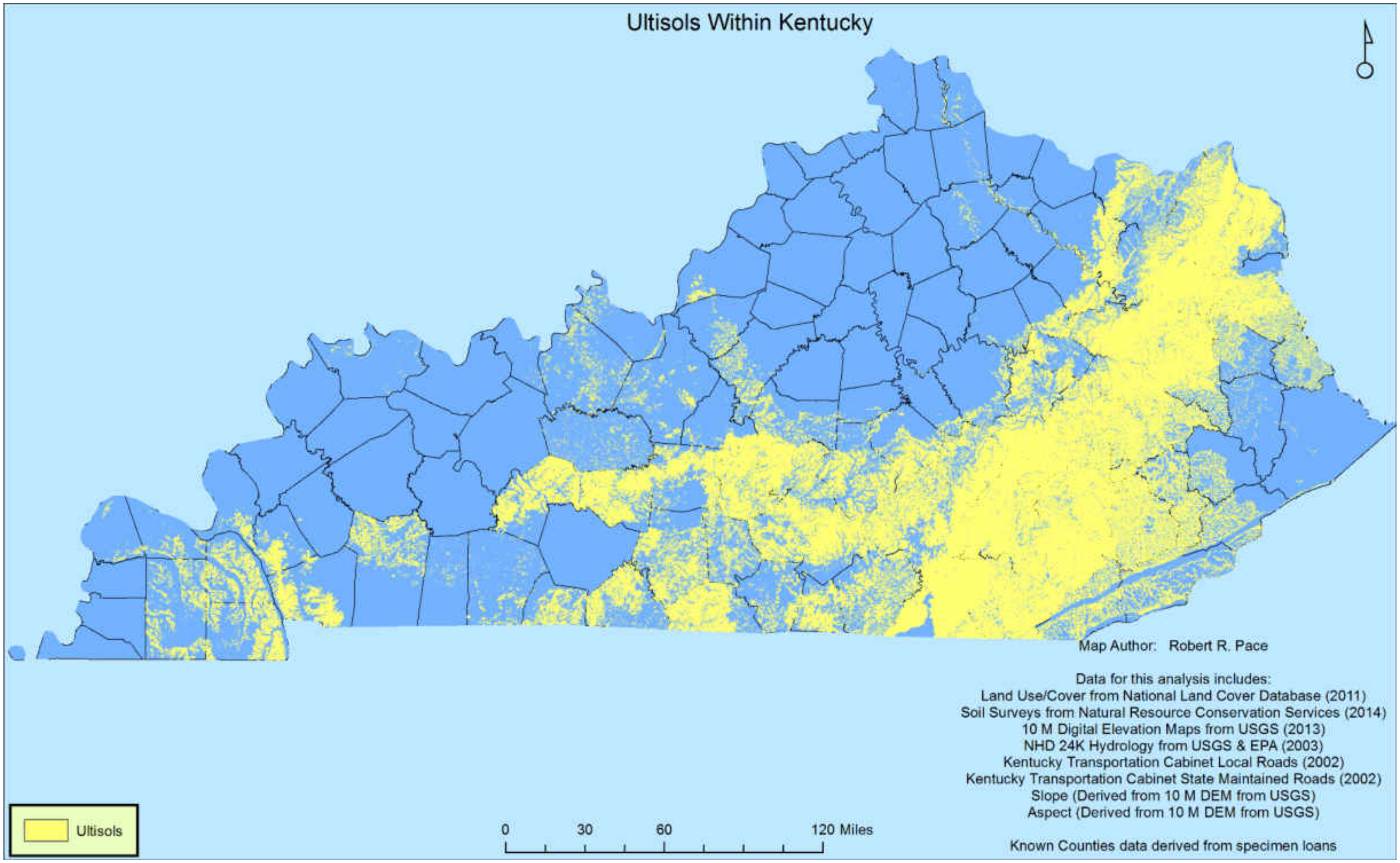


Figure 46. Map of Kentucky showing the distribution of ultisols within the state.

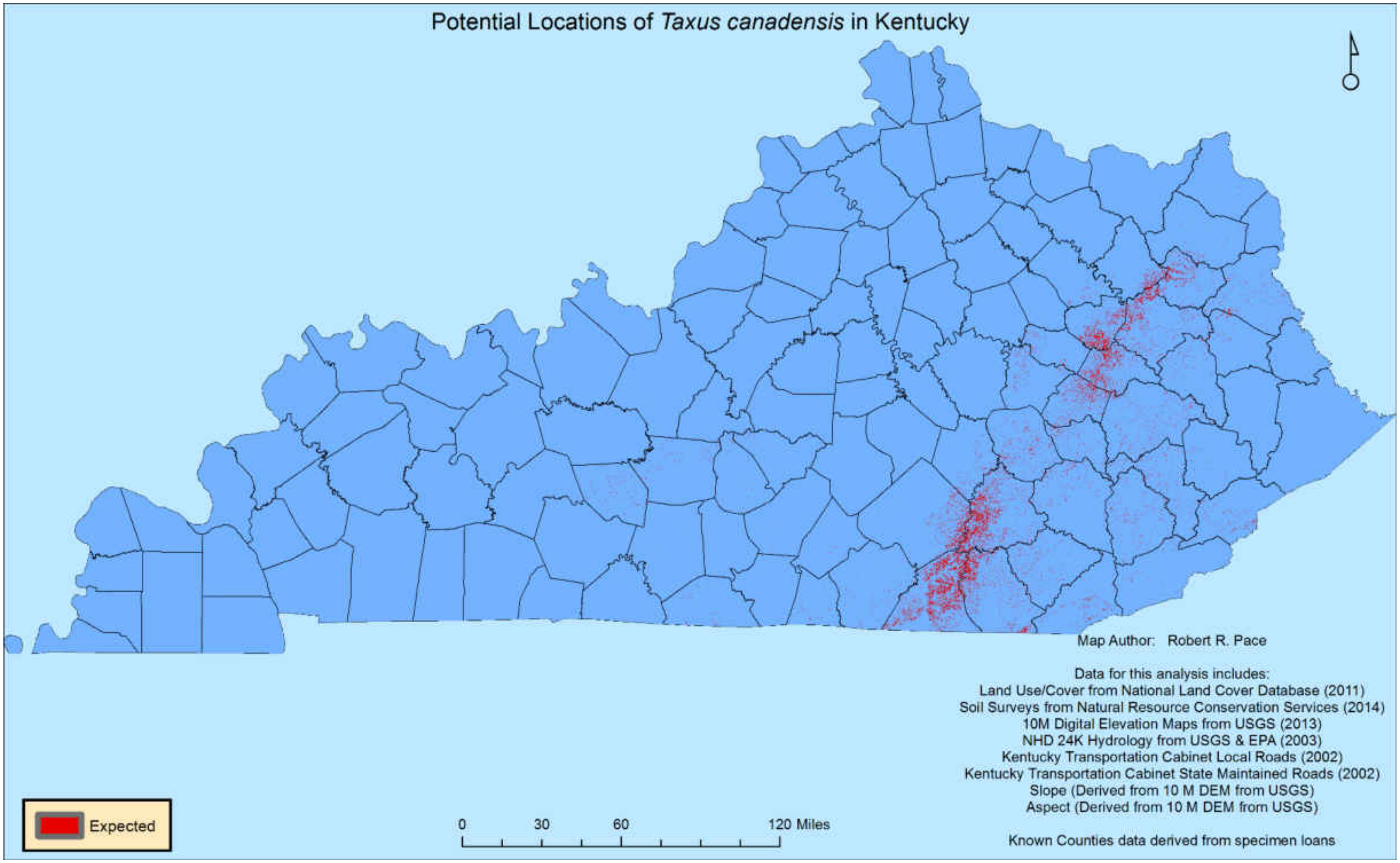


Figure 47. Map of the Kentucky showing potential distribution of *Taxus canadensis*

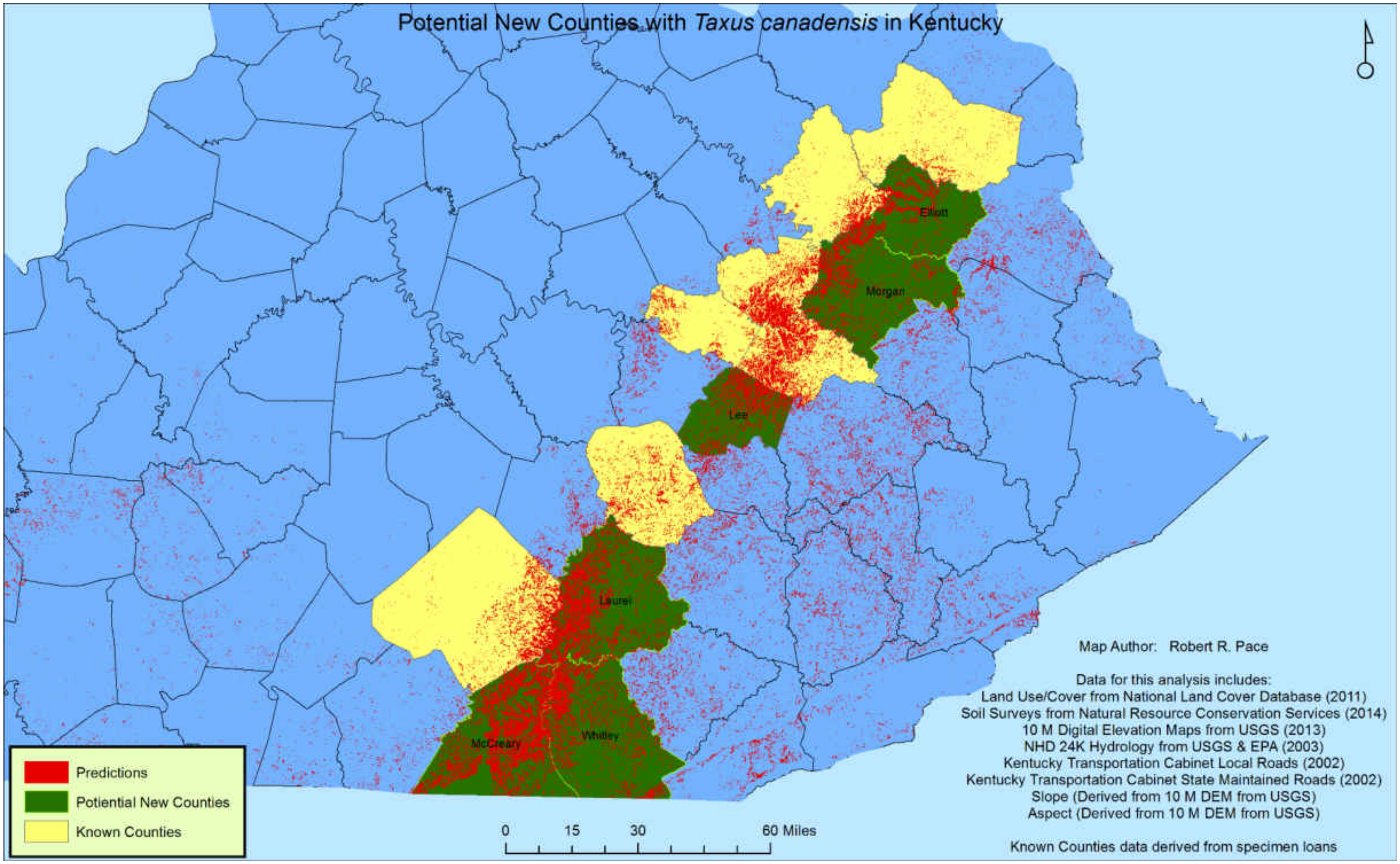


Figure 48. Map of Eastern Kentucky showing both known and predicted distribution

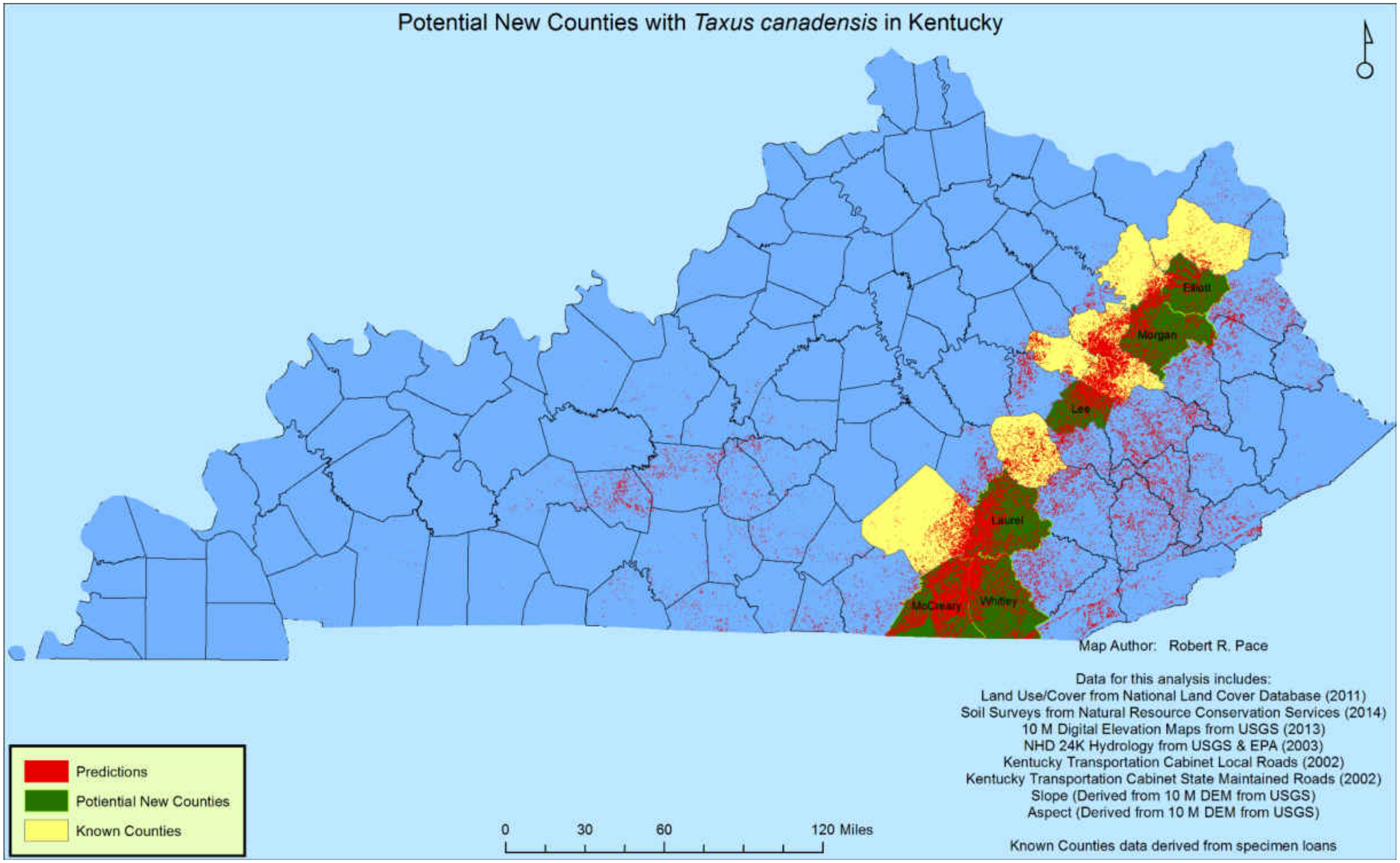


Figure 49. Map of Kentucky showing known and predicted distribution of *Taxus canadensis* derived from herbarium specimens and habitat modeling.

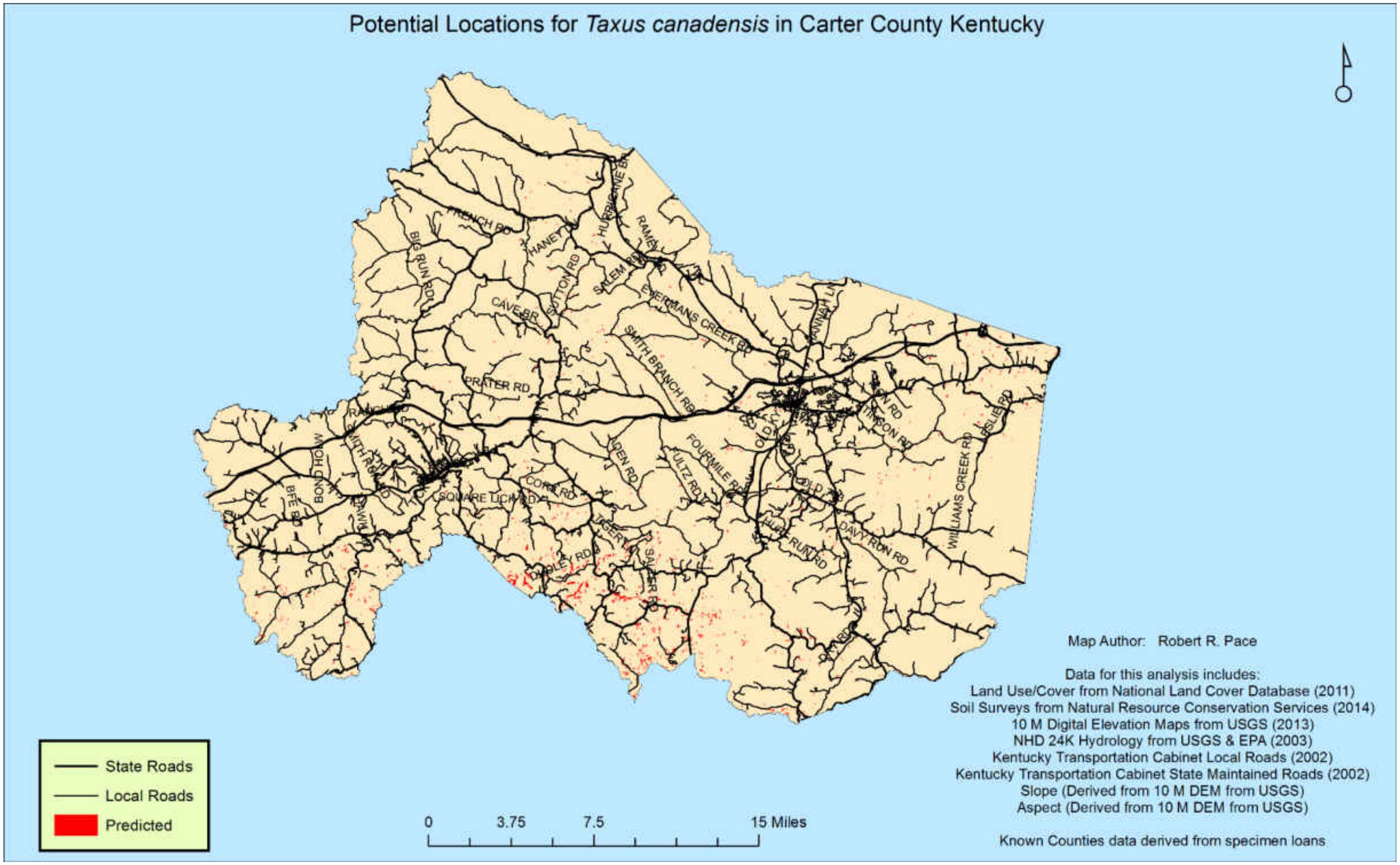


Figure 50. Map of Carter county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.

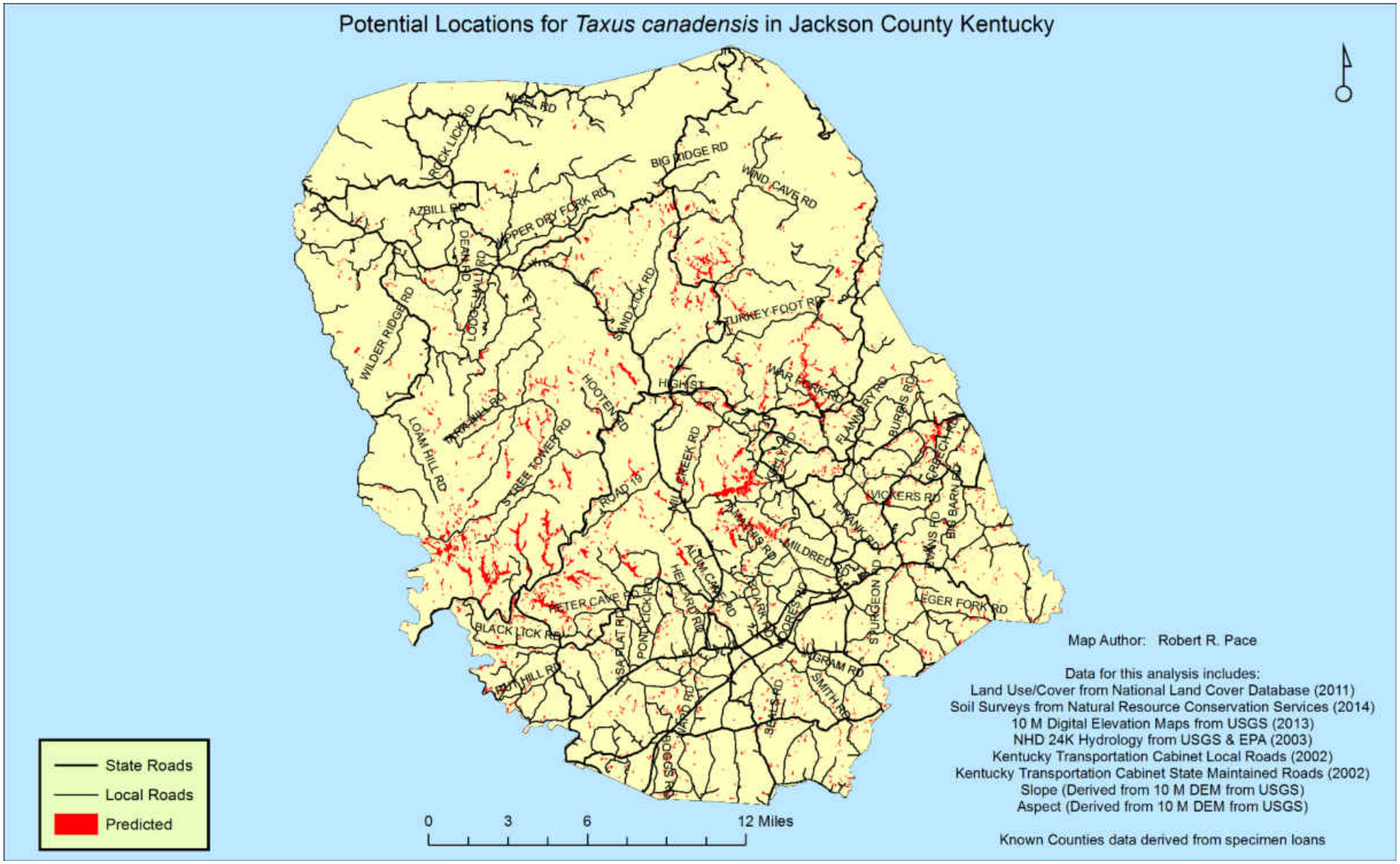


Figure 51. Map of Jackson county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.

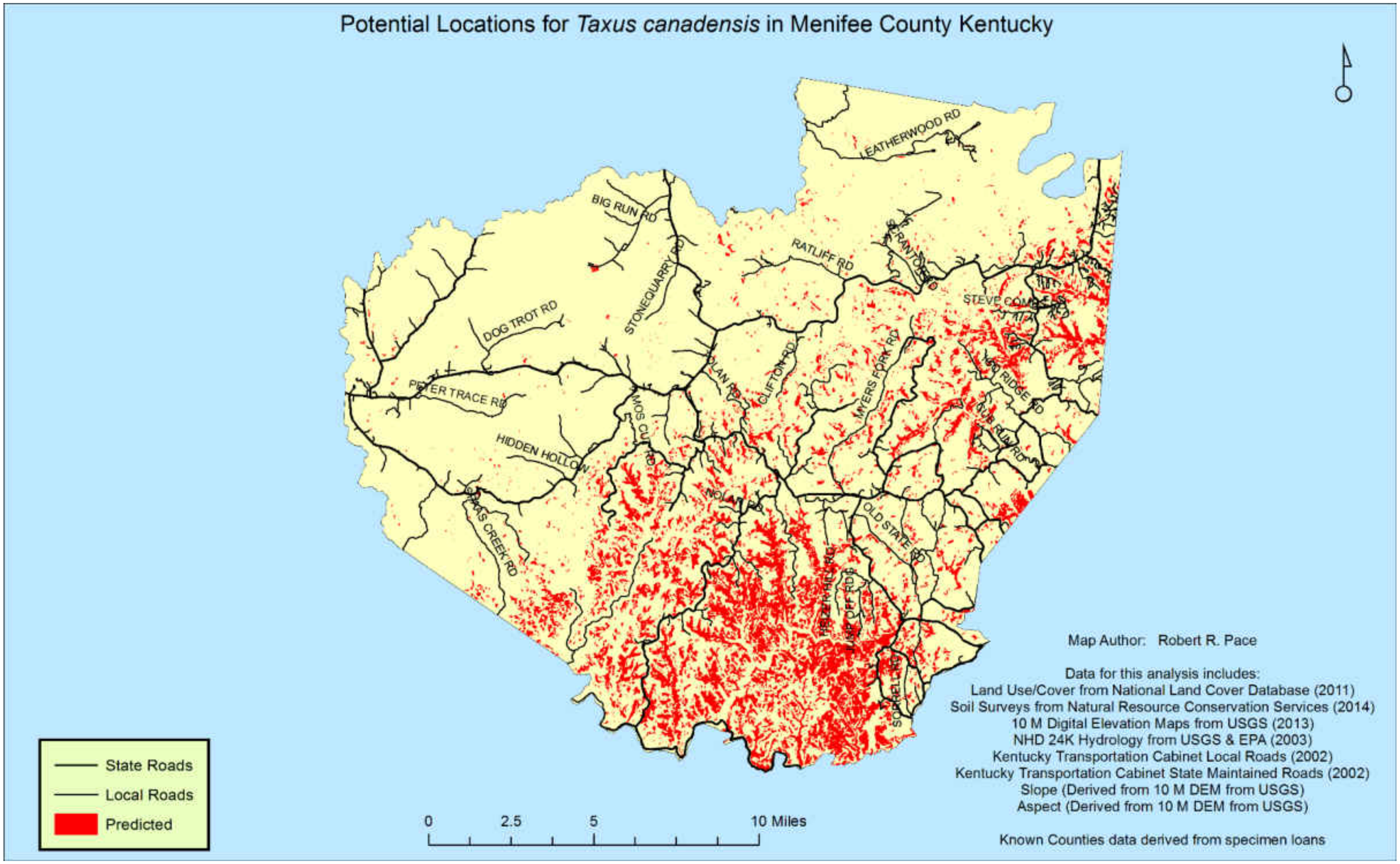
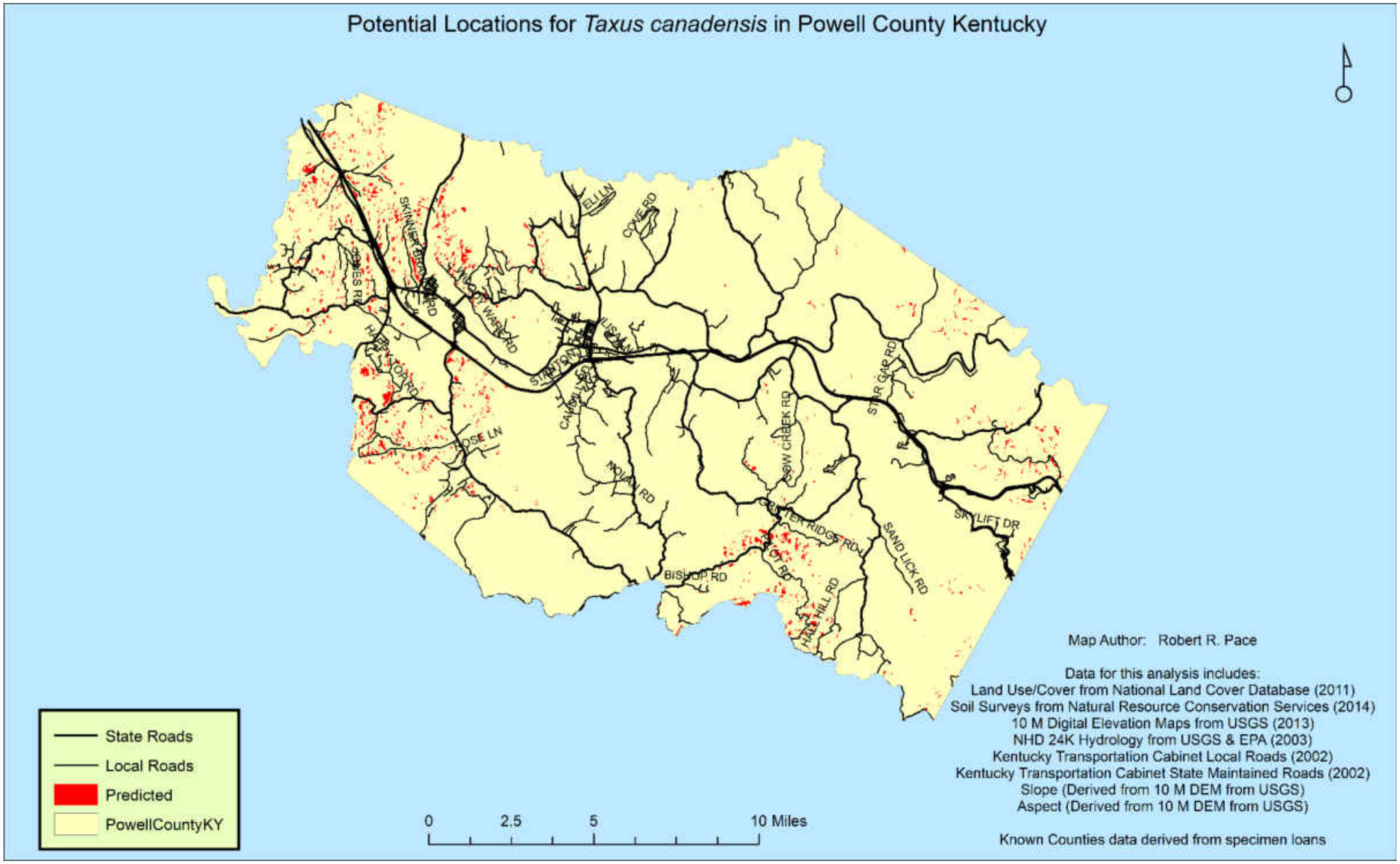


Figure 52. Map of Menifee county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.


Potential Locations for *Taxus canadensis* in Powell County Kentucky

Map Author: Robert R. Pace

Data for this analysis includes:
Land Use/Cover from National Land Cover Database (2011)
Soil Surveys from Natural Resource Conservation Services (2014)
10 M Digital Elevation Maps from USGS (2013)
NHD 24K Hydrology from USGS & EPA (2003)
Kentucky Transportation Cabinet Local Roads (2002)
Kentucky Transportation Cabinet State Maintained Roads (2002)
Slope (Derived from 10 M DEM from USGS)
Aspect (Derived from 10 M DEM from USGS)

Known Counties data derived from specimen loans

Figure 53. Map of Powell county Kentucky showing potential locations of *Taxus canadensis d*erived from habitat modeling.

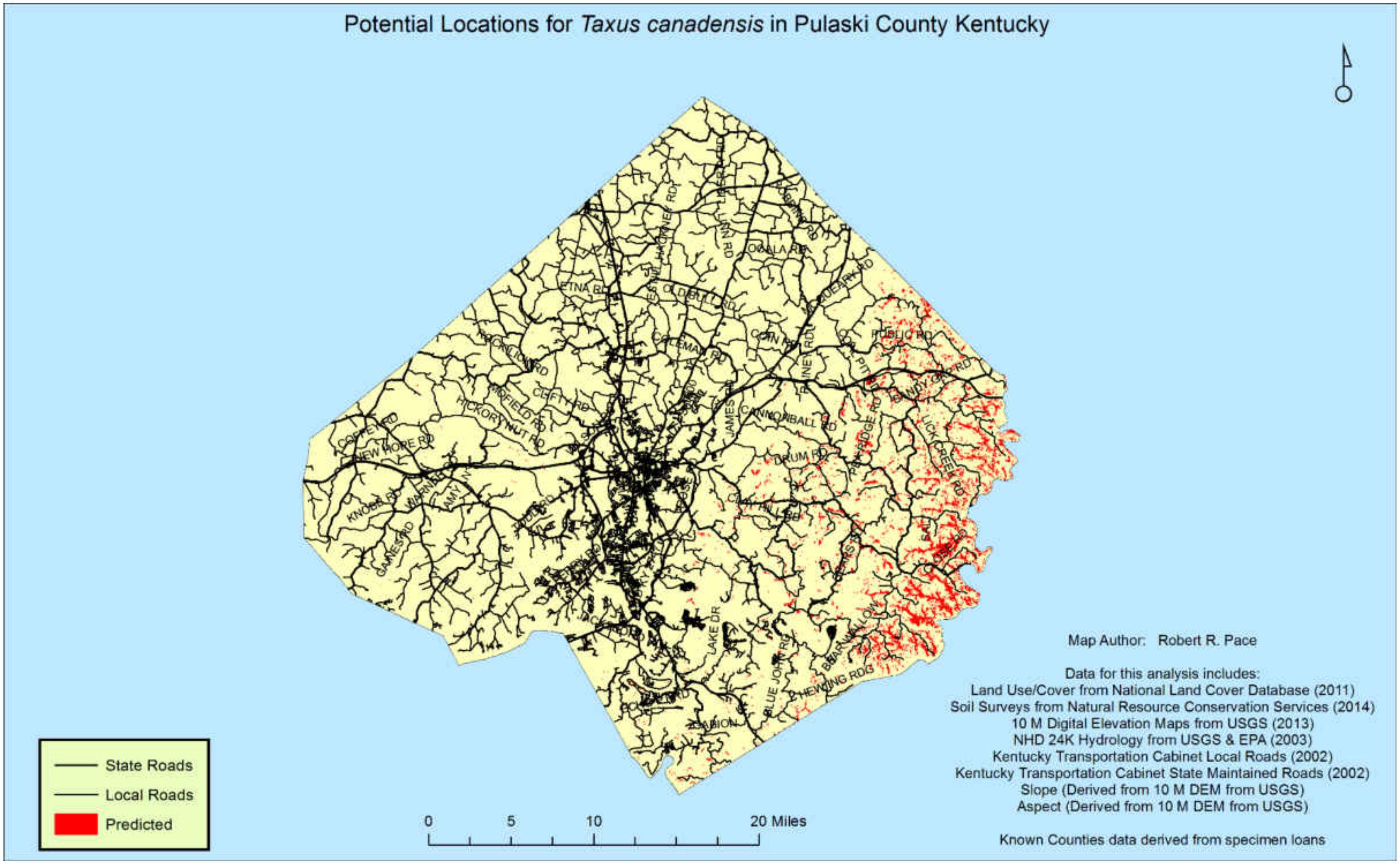


Figure 54. Map of Pulaski county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.

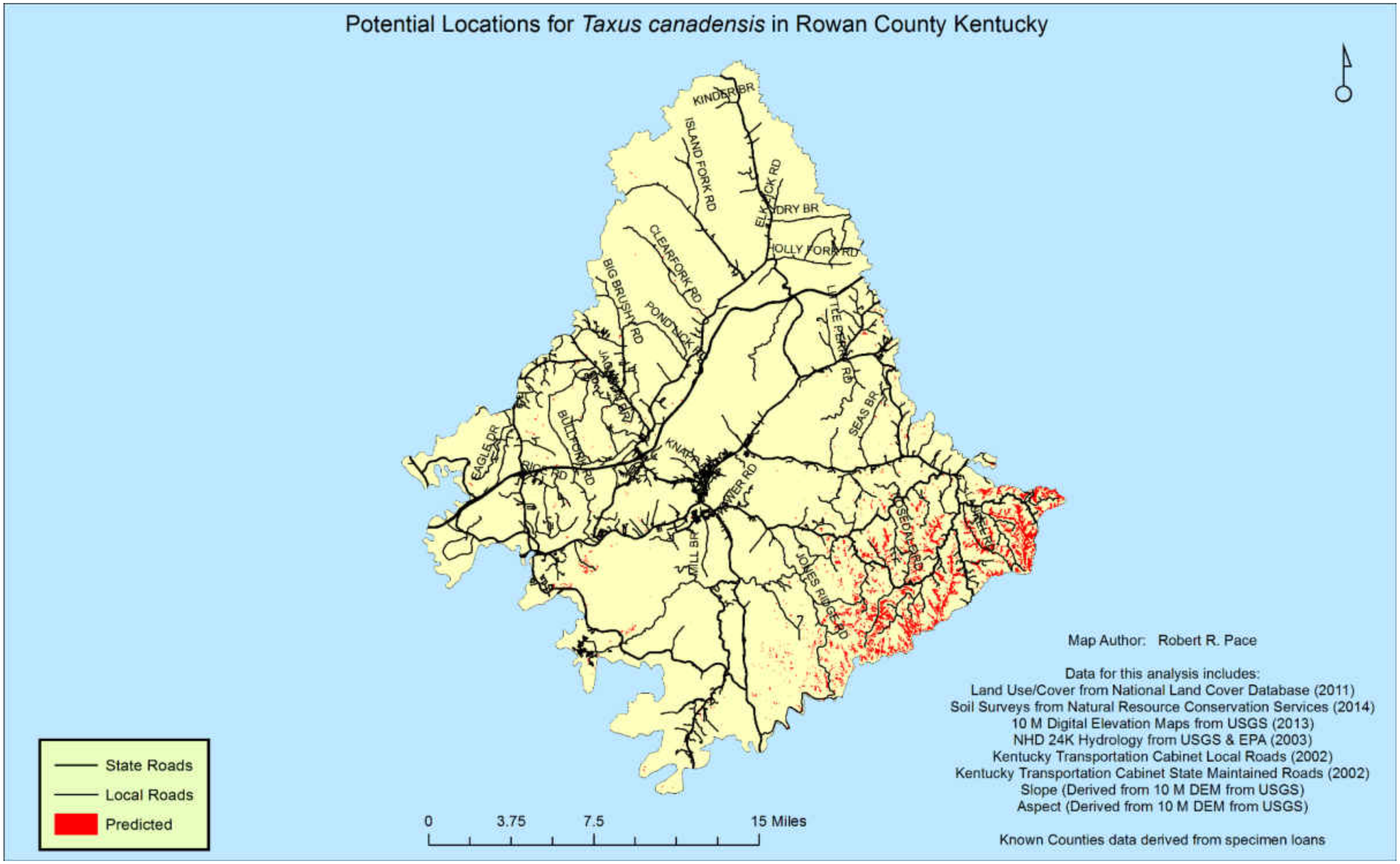


Figure 55. Map of Rowan county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.

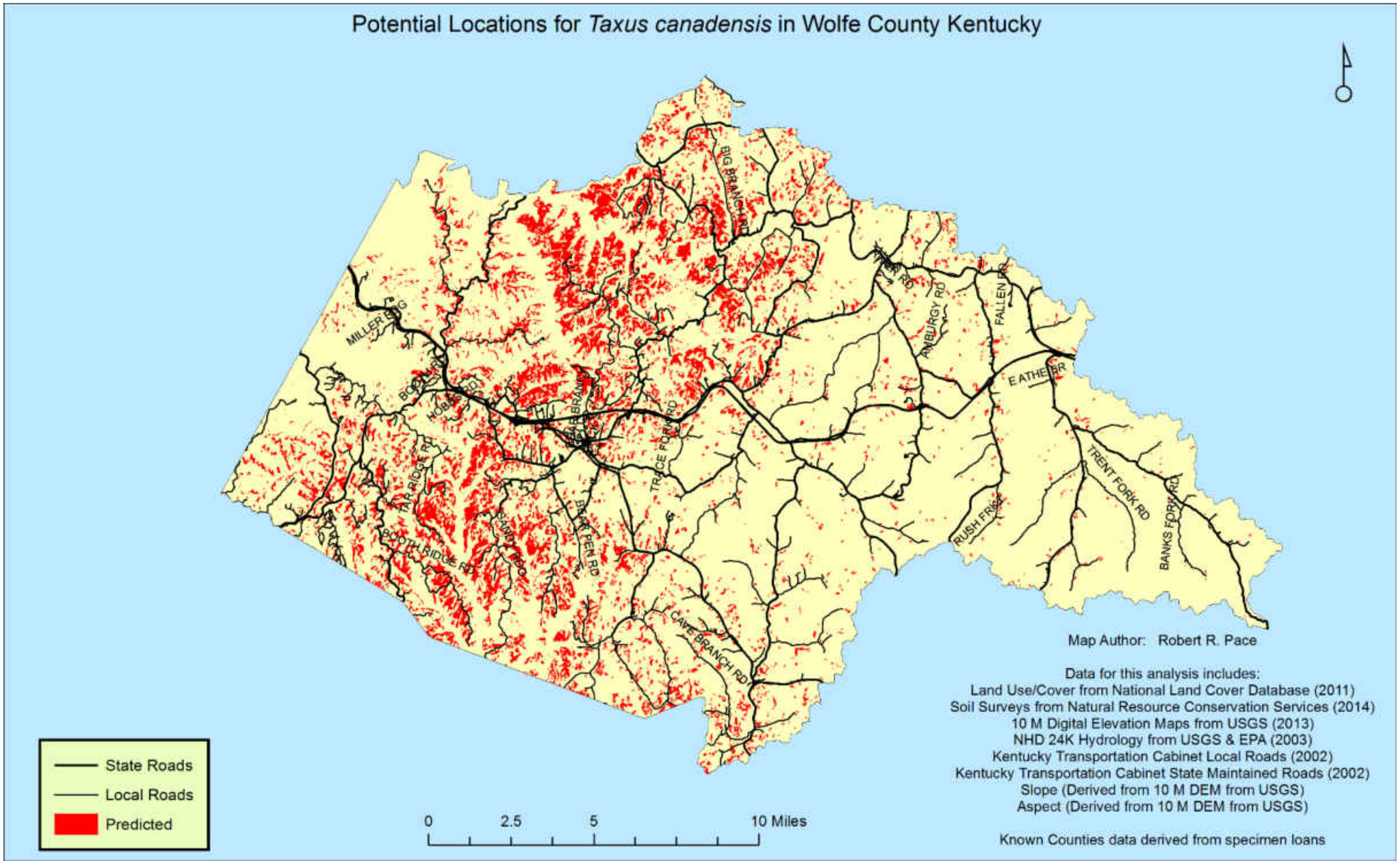


Figure 56. Map of Wolfe county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.

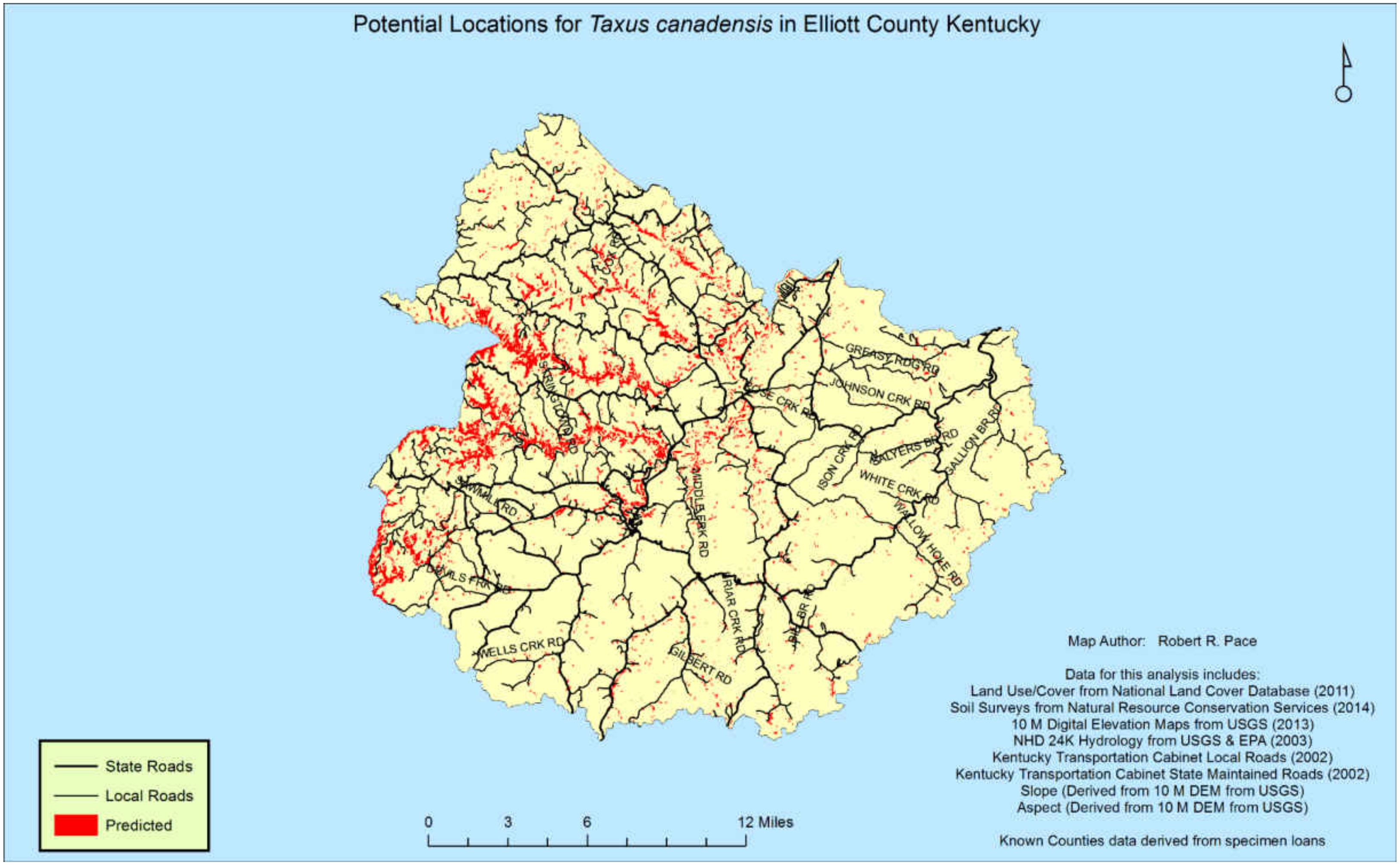


Figure 57. Map of Elliott county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.

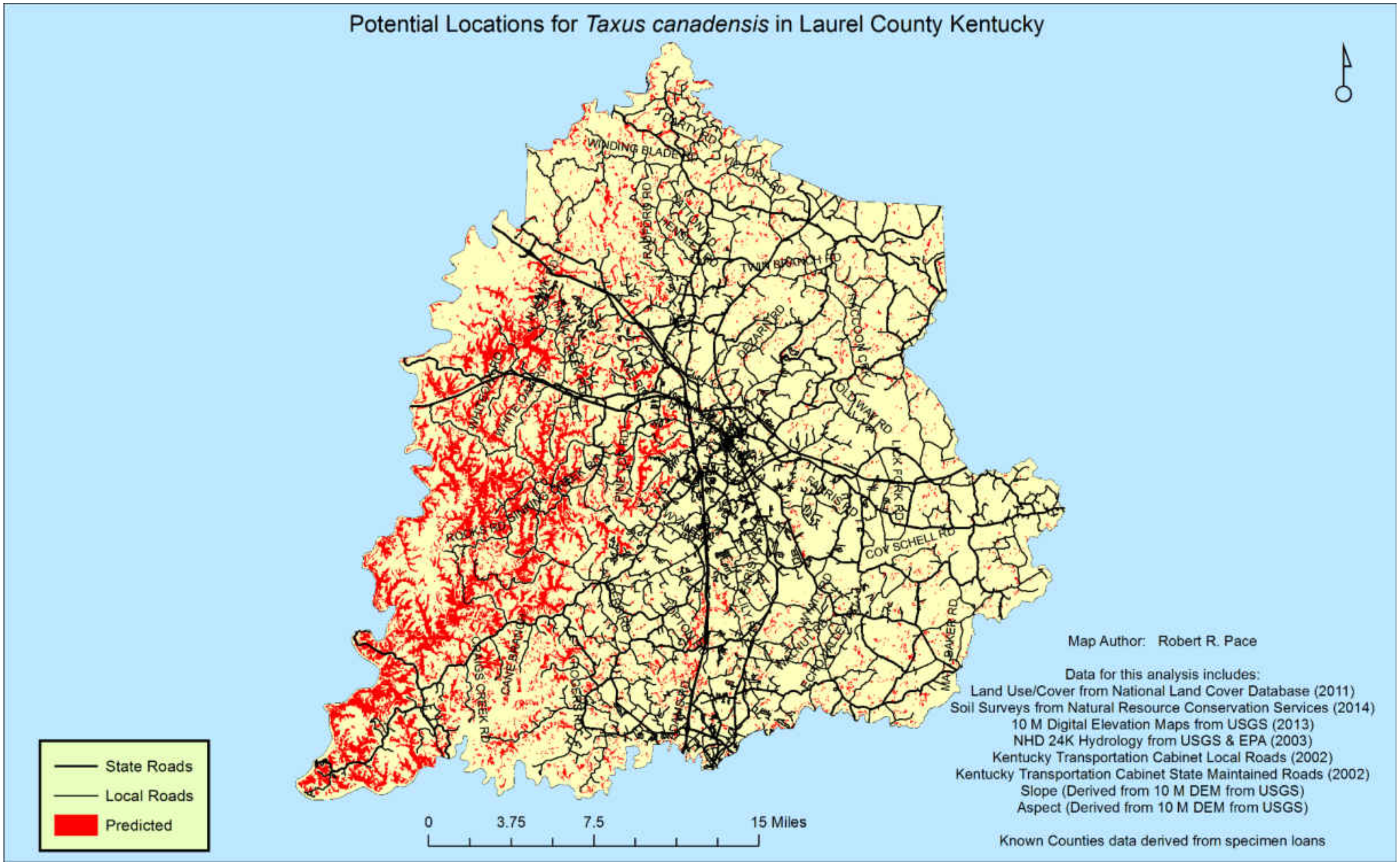


Figure 58. Map of Laurel county Kentucky showing potential locations of *Taxus canadensis d*erived from habitat modeling.

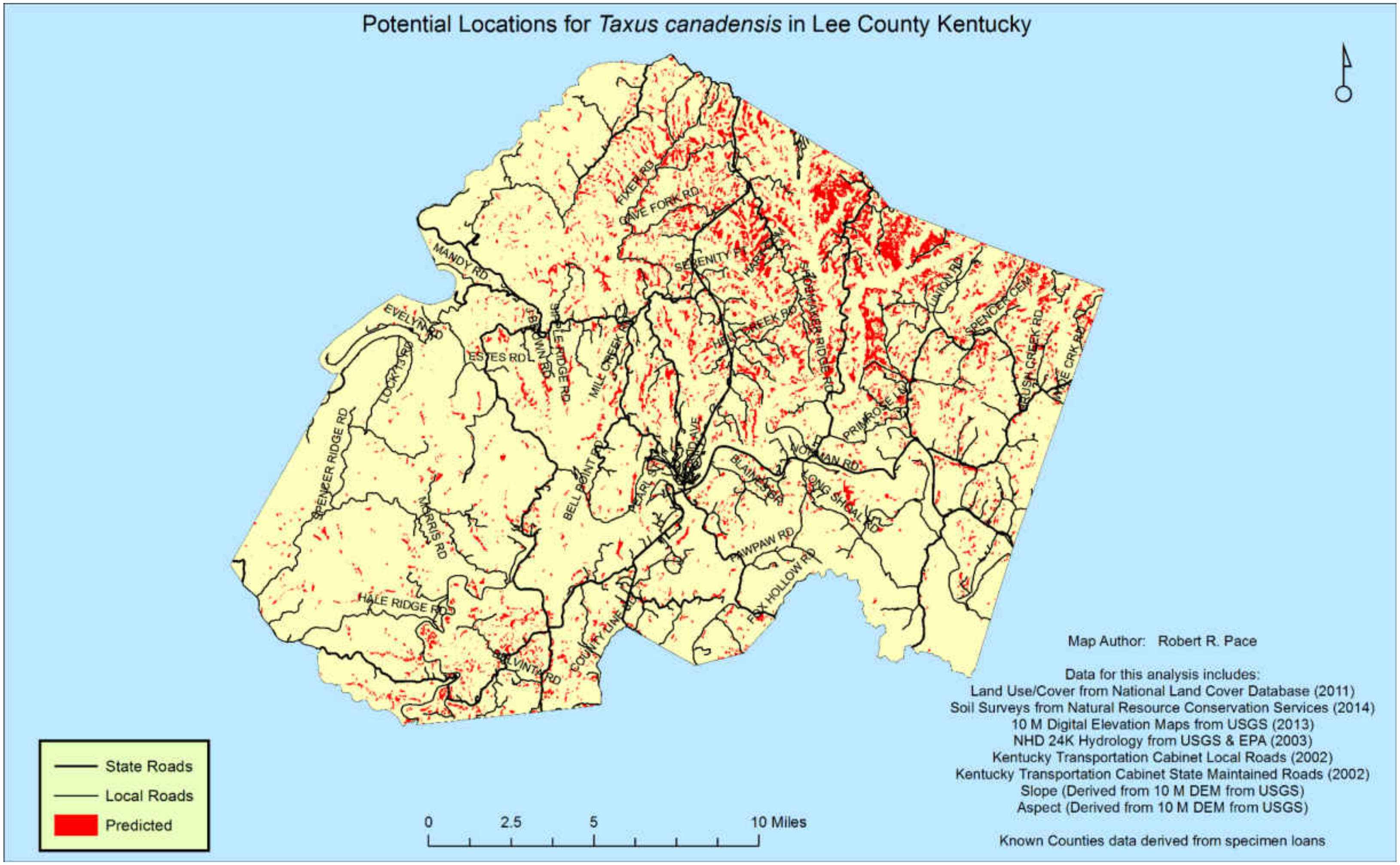
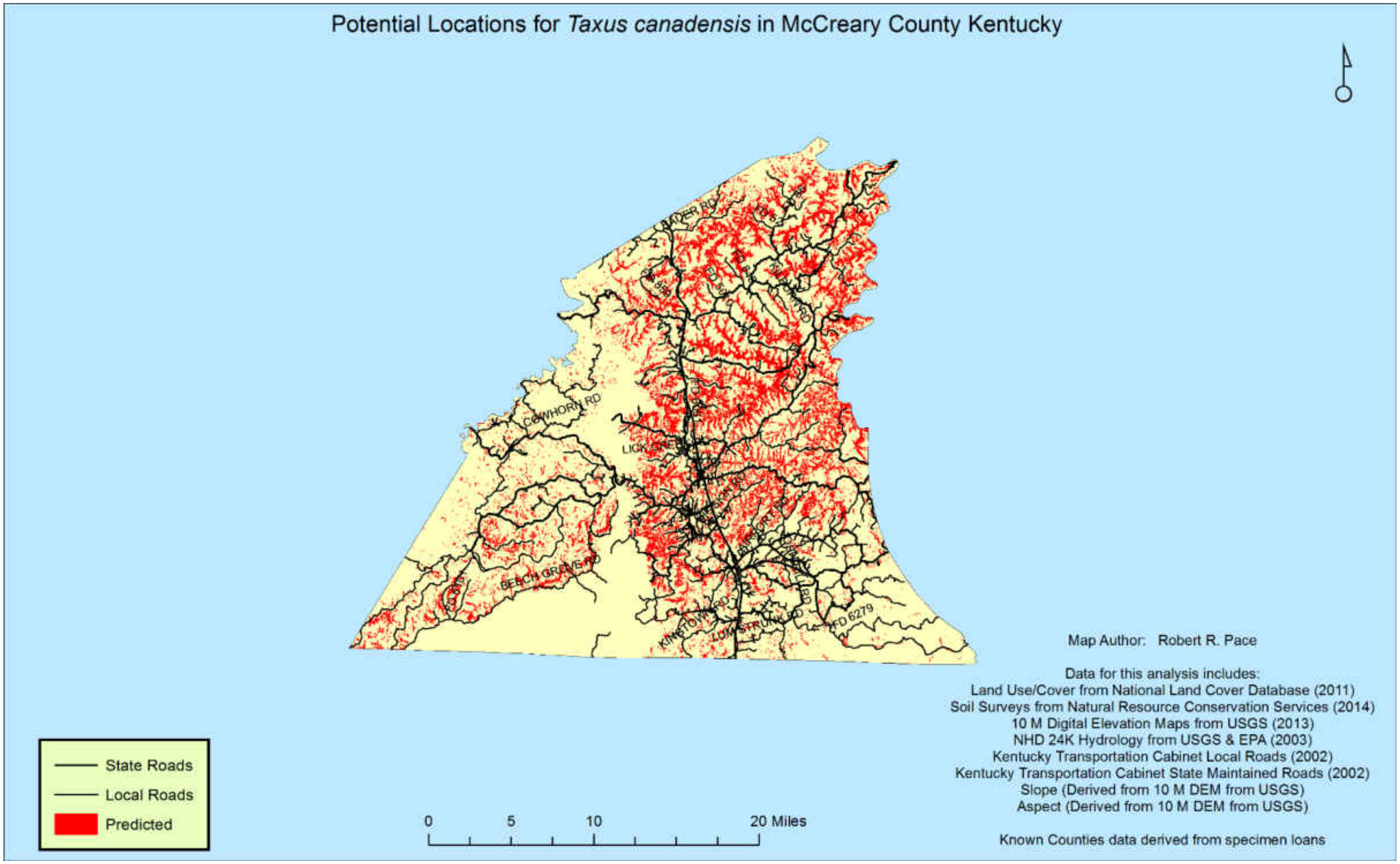


Figure 59. Map of Lee county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.



Map Author: Robert R. Pace

Data for this analysis includes:
Land Use/Cover from National Land Cover Database (2011)
Soil Surveys from Natural Resource Conservation Services (2014)
10 M Digital Elevation Maps from USGS (2013)
NHD 24K Hydrology from USGS & EPA (2003)
Kentucky Transportation Cabinet Local Roads (2002)
Kentucky Transportation Cabinet State Maintained Roads (2002)
Slope (Derived from 10 M DEM from USGS)
Aspect (Derived from 10 M DEM from USGS)

Known Counties data derived from specimen loans

Figure 60. Map of McCreary county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.

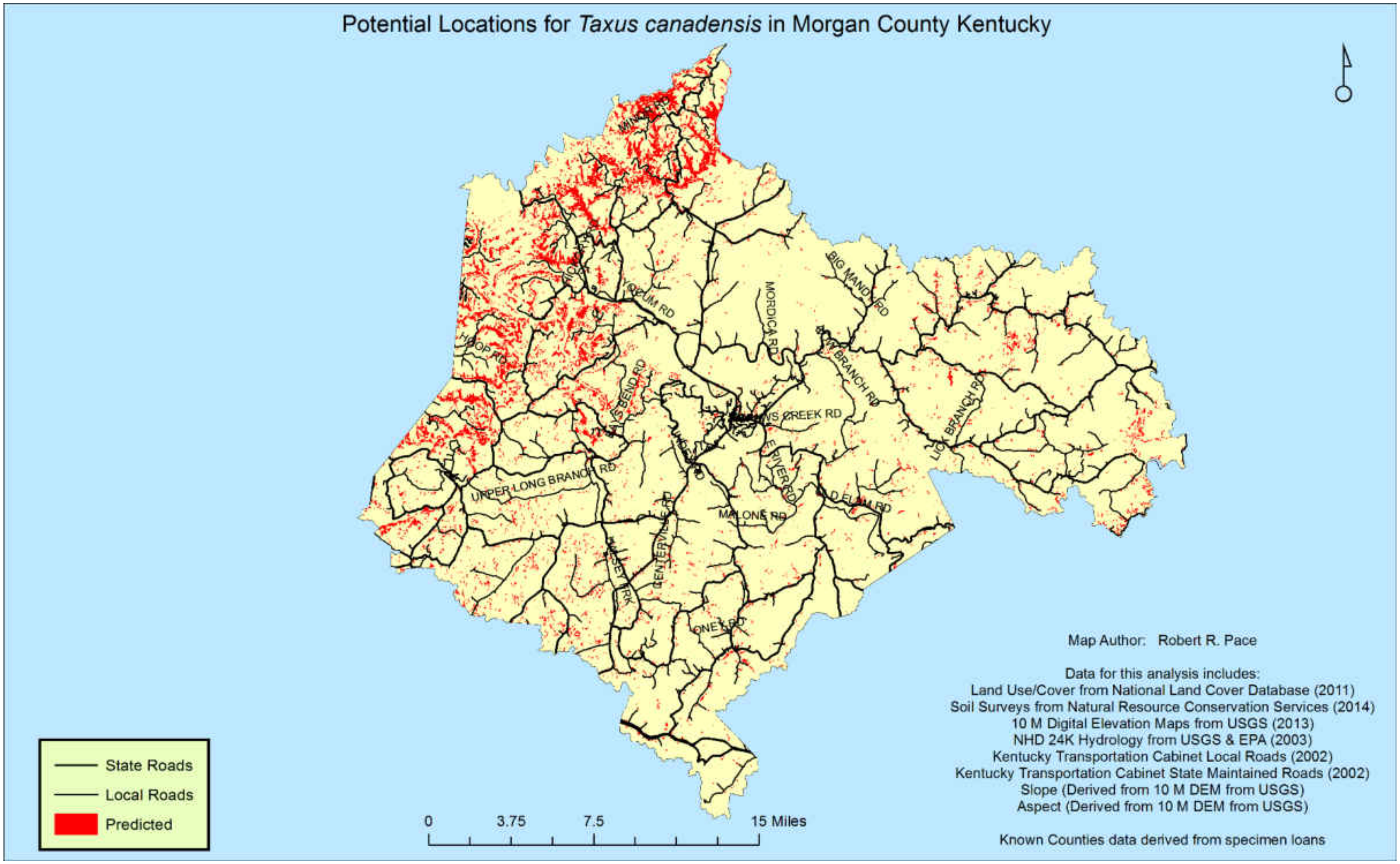
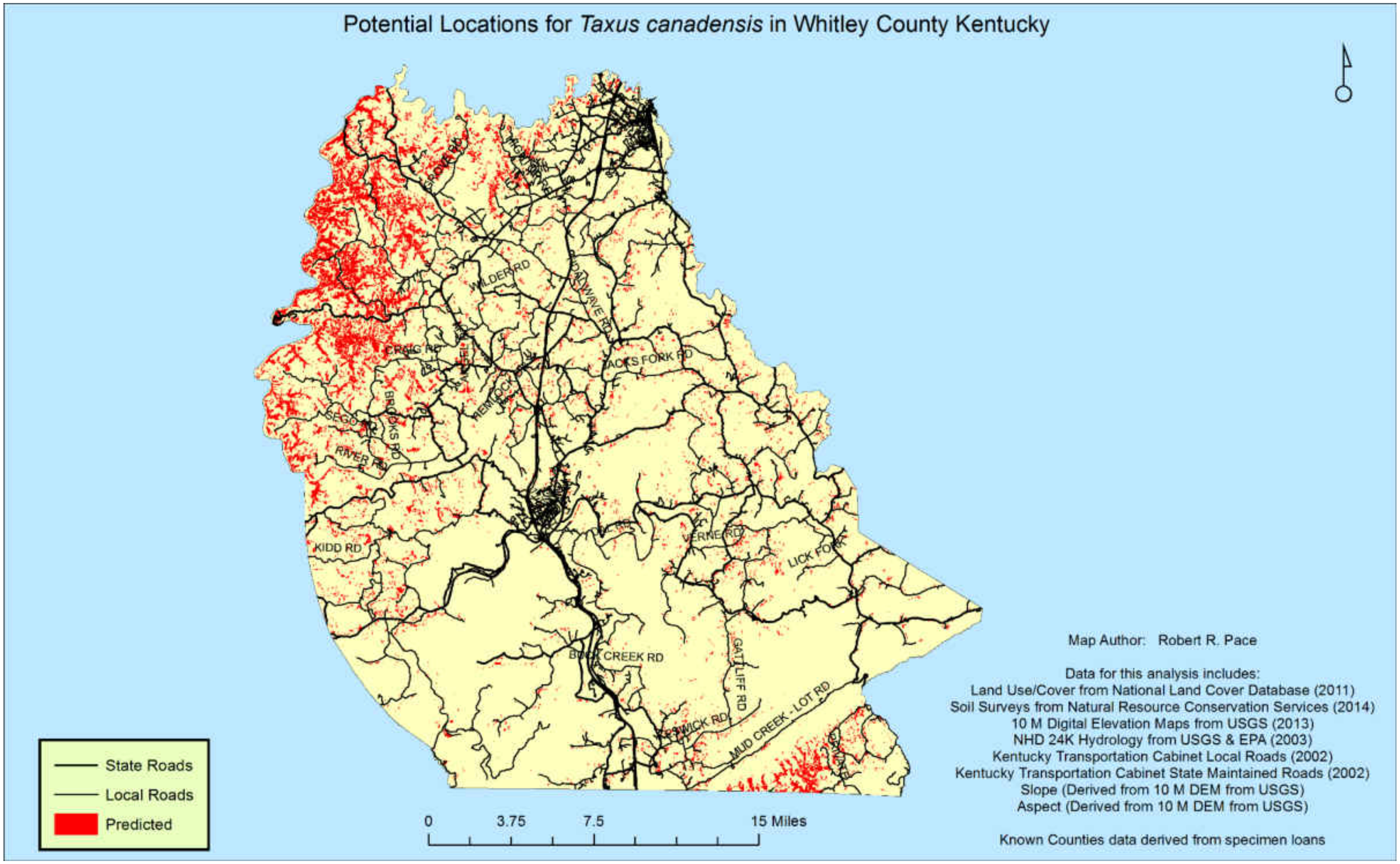


Figure 61. Map of Morgan county Kentucky showing potential locations of *Taxus canadensis d*erived from habitat modeling.



Potential Locations for *Taxus canadensis* in Whitley County Kentucky

Map Author: Robert R. Pace

Data for this analysis includes:
Land Use/Cover from National Land Cover Database (2011)
Soil Surveys from Natural Resource Conservation Services (2014)
10 M Digital Elevation Maps from USGS (2013)
NHD 24K Hydrology from USGS & EPA (2003)
Kentucky Transportation Cabinet Local Roads (2002)
Kentucky Transportation Cabinet State Maintained Roads (2002)
Slope (Derived from 10 M DEM from USGS)
Aspect (Derived from 10 M DEM from USGS)

Known Counties data derived from specimen loans

Figure 62. Map of Whitley county Kentucky showing potential locations of *Taxus canadensis* derived from habitat modeling.

APPENDIX F: Specimen Images

Figure 63. Specimen image of *Taxus baccata* ‘repandens’ obtained from Northern Kentucky University’s Herbarium (KNK).

Herbarium of Northern Kentucky University (KNK)

PLANTS OF OREGON

Taxus brevifolia

Tree 30 ft tall, ca. 10 inches DBR.
Seed (incl. "cup") depressed-globose, ca 1/3 inch wide.
"Cup" translucent orange-red, seed pale brown.

Elev. 4700 ft in Cascade Range.

Ca. 20 miles E of Medford along highway 140.
County: JACKSON
Date: 10 AUG 1986
Matthew H. Hils
John W. Thieret

Figure 64. Specimen image of *Taxus brevifolia* obtained from Northern Kentucky University's Herbarium (KNK).

AUSTIN PEAY STATE UNIVERSITY HERBARIUM (APSC)
CLARKSVILLE, TENNESSEE, U.S.A.

Plants of Pennsylvania
Erie County

Taxaceae

Taxus canadensis Marsh.

Six Mile Creek Park, E side of Erie, along Sixmile Creek 150 ft upstream from Depot Rd (Hwy 531) bridge on S side of creek. Moist, shady N-facing lower slopes above creek. Several individuals about 2 ft tall.

42.122754° Latitude, -79.921764° Longitude.

May 2010

Dwayne Estes with Matt Bruton and Kim Norton

No.: 11566

Figure 65. Specimen image of *Taxus canadensis* obtained from University of Tennessee Herbarium (TENN).

Figure 66. Specimen image of *Taxus cuspidata* obtained from West Virginia University's Herbarium (WVU).

Figure 67. Specimen image of *Taxus floridana* obtained from Northern Kentucky University's Herbarium (KNK).

Figure 68. Specimen image of *Taxus globosa* obtained from Northern Kentucky University's Herbarium (KNK).

Herbarium of Northern Kentucky University (KNK)

PLANTS OF OHIO

Taxus hunnewelliana A31-197

Shrub to 10 ft

Cultivated in the "Living Herbarium of Taxus" at the Secrest Arboretum, Ohio Agricultural Research and Development Center, Wooster.

County: WAYNE
Date: 9 November 1991 John W.Thieret

SOTIKY-023-KNK023

Figure 69. Specimen image of *Taxus* × *hunnewelliana* obtained from Northern Kentucky University's Herbarium (KNK).

CULTIVATED PLANTS OF
THE PEOPLE'S REPUBLIC OF CHINA
JIANGSU PROVINCE
Nanjing

Taxus mairei (Lem.& Levl.) S.Y. Hu ex Liu

Triple-trunked, small ascending tree; 2-3.5 m tall; DBH 2-10 cm; bark reddish and flaky, revealing green beneath flakes; branches arcuate; lvs. glossy dark green above, yellowish-green below; fls. immature, female, dark green.
Cultivated in the Nanjing Botanical Garden Memorial Sun Yat-Sen of the Jiangsu Institute of Botany.
Note: This species is very dramatic in appearance and should be introduced to the U.S.

Coordinates: 32°03'N, 118°47'E

Coll. SAPSBE Coll. No. 45324 Coll. Date 25 Jun 1987

SINO-AMERICAN PURPLE SHAN BOTANICAL EXPEDITION (SAPSBE)
T.R. Dudley (NA), E.C. Dudley (YU), Yang Z.B. (NAS),
and Sun Ming-jun (NAS)

U. S. NATIONAL ARBORETUM HERBARIUM, WASHINGTON, D. C.

Figure 70. Specimen image of *Taxus mairei* obtained from University of Tennessee Herbarium (TENN).

Herbarium of Northern Kentucky University (KNK)

PLANTS OF OHIO

Taxus media 'Coleana'
shrub ca. 6 ft tall

A30-131

leaf
arrangement

Cultivated in the"Living Herbarium of Taxus" at the Secrest Arboretum, Ohio Agricultural Reserach and Development Center, Wooster.

County: WAYNE
Date: 9 November 1991

John W.Thieret

Figure 71. Specimen image of *Taxus* × *media* ‘coleana’ obtained from Northern Kentucky University’s Herbarium (KNK).