

# *Botrychium multifidum*

Leathery Grape Fern  
Ophioglossaceae



Photograph by Katherine Zacharkevics (Anderson, 2005)

## *Botrychium multifidum* Rare Plant Profile

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## Introduction

The life cycle of ferns warrants a brief review. The familiar image of a fern frond is the sporophyte; it is diploid, larger, green, and leafy. The sporophyte produces spores instead of seeds. Spores germinate into the gametophyte, which is haploid, tiny, and may be terrestrial or subterranean depending on the species. When mature, the gametophyte produces gametes – male and female sex cells. Male gametes move through soil water to fertilize female gametes; successful fertilization produces the next sporophyte, thus completing the cycle – the alternation of generations (Fig. 1). In ferns, the gametophyte lives independently of the sporophyte (Anderson, 2005); these are two distinct life phases, each potentially having different ecological requirements.

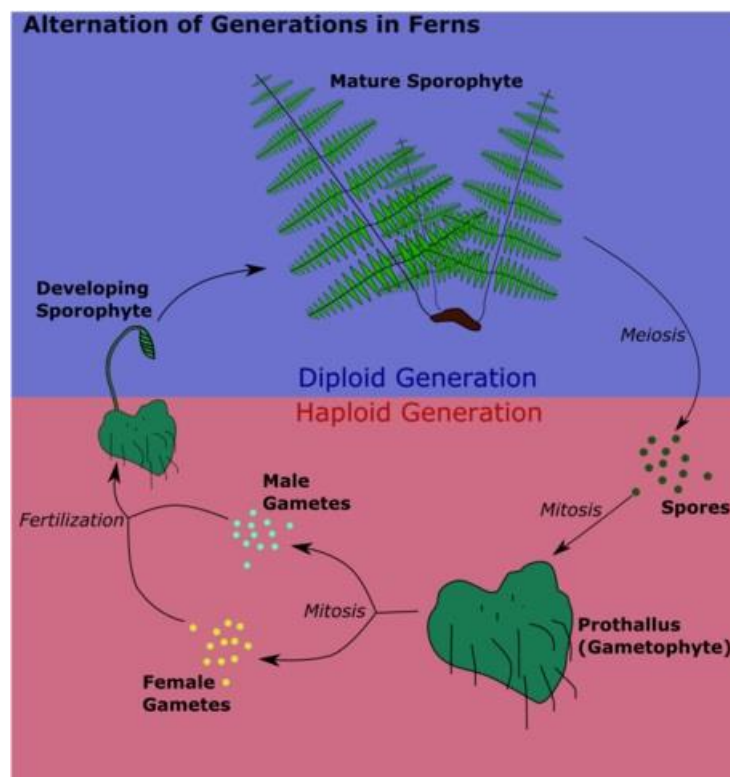


Figure 1. Diagram of a fern life cycle. ©Jeffrey Finkelstein

Limited research is devoted solely to the biology, ecology, and management of *Botrychium multifidum*. Where information about *B. multifidum* is lacking, we include general information for the genus *Botrychium* or other *Botrychium* species, with the understanding that specifics for *B. multifidum* may differ.

## Life History

The genus *Botrychium* is split into three subgenera; *Botrychium multifidum* is in the subgenus *Sceptridium*. The subgenus *Sceptridium* is further split into two sections; *B. multifidum* is in the section *Sceptridium* (Flora of North America, 2020).

### *Sporophyte morphology*

The fern life cycle is characterized by the alternation of generations, described above in the Introduction; the conspicuous form is called the sporophyte and the inconspicuous form is the gametophyte.

The sporophyte is composed of two parts connected to a common stalk: a photosynthetic sterile leaf called the trophophore and a fertile spore-bearing structure called the sporophore (Figure 2A). Leaves appear in late spring and stay green over winter. Wagner (1960) reported that *B. multifidum* leafed out in late April/early May in southern Michigan. The leaf may take three or four months to fully unfurl (Anderson, 2005). *Botrychium multifidum* usually produces only one leaf per year, but occasionally may produce two (Stevenson, 1975; Mesipuu et al., 2009). The leaf remains green through the winter and may still be visible yet withered or yellowed the following summer as the new frond emerges (Mesipuu et al., 2009).

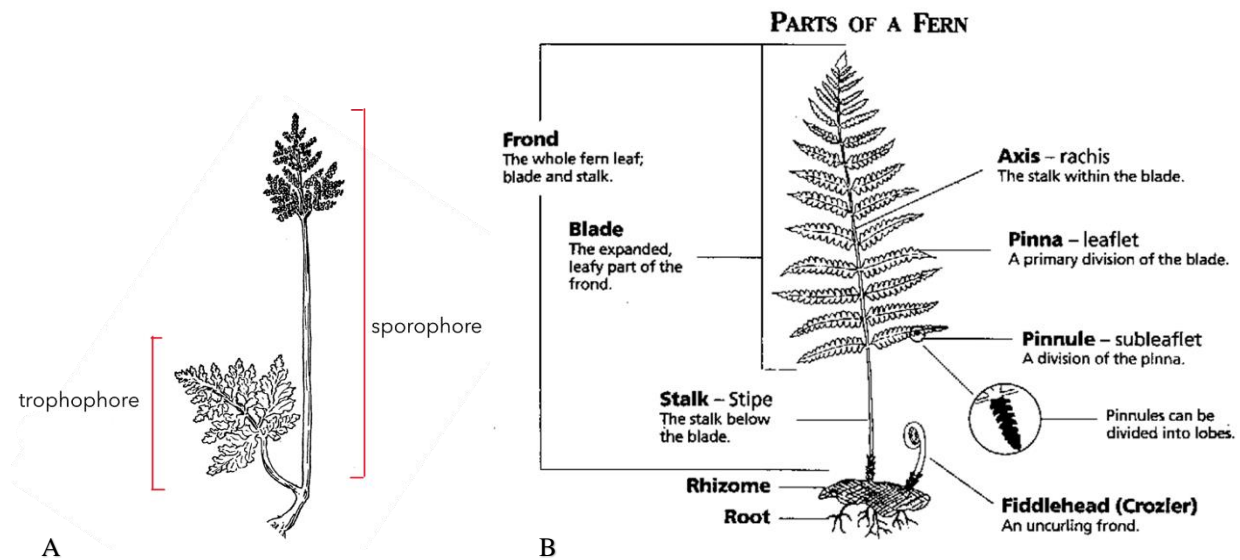


Figure 2. A) *Botrychium* spp. trophophore and sporophore (USDA-NRCS PLANTS Database / Britton, N.L., and A. Brown, 1913); B) Generic fern morphology (Bowe, 2013).

The sterile leaf stalk is 2-15 cm long, erect or ascending, 0.3-1.2 times the length of the rachis (Flora of North America, 2020). The leaf sheaths are closed. The sterile leaf blade is triangular bipinnate or tripinnate, 6 to 30 cm long, and 3.5 to 20 cm wide, shiny green, with a leathery texture. The pinnae are horizontal to ascending; the distance between first and second pinnae is equal to or slightly more than between the second and third pairs. The pinnae are divided to the tip. The basal pinnae are short-stalked and mostly subopposite (Anderson, 2005; Flora of North America, 2020).

The pinnules are ovate with a rounded tip; the margins are usually entire to shallowly crenulate (sometimes inconspicuously and shallowly denticulate). The terminal pinnules are similar to or only slightly larger than lateral pinnules (Flora of North America, 2020). The chief terminal divisions are not elongate, as for *B. dissectum* (Clausen and Edwards, 1937). The venation pattern is pinnate with a strong midrib (Flora of North America, 2020).

*B. multifidum* doesn't reproduce vegetatively through gemmae as some other species in this genus do (Mesipuu et al., 2009), so it must rely on spore production. Spores are produced on the sporophore, which is connected to the sterile frond on the basal portion of a shared stalk. *Botrychium* species in subgenus *Sceptridium* – including *B. multifidum* – don't necessarily produce a sporophore every year; dormancy is fairly common (Anderson, 2005).

The sporophore (fertile) stalks and midrib are slightly flattened, not fleshy, 2-3-pinnate, and 1.2 times the length of the sterile leaf (Flora of North America, 2020), or 8 to 40 cm long (Anderson, 2005). Sporangia clusters resemble grape clusters (Olejnik and Celka, 2015). As the spores mature in August or September (Clausen and Edwards, 1937), the sporangia change color from green to yellow to brown (Wagner, 1960).

*Botrychium multifidum* has up to 15 blackish colored roots (Flora of North America, 2020) that do not have root hairs (Anderson, 2005). *B. multifidum* has contractile roots which keeps the bud at an optimal location beneath the soil surface as the root grows (Stevenson, 1975). *Botrychium multifidum* is unique among ferns in having this adaptation that is more commonly seen in higher plants (Baas-Becking, 1921; Braggins, 1980; Ahlenslager and Potash, 2007). The root may send up multiple closely spaced ramets, which from above the soil surface appear to be individual plants (Stevenson, 1975).

#### *Size changes across range*

Morphological variation along environmental gradients is common for *Botrychium* (Braggins, 1980; Wagner, 1962), but size doesn't seem to be related to age (Anderson, 2005). At a large scale, *B. multifidum* expresses changes in size along a latitudinal gradient; the smallest plants are found north of 45°N, intermediate sized plant center between 40-42°N; and the largest specimens are found in the south, at roughly 37°N (Wagner, 1962). For example, in California the fronds are often 20 to 35 cm wide (Anderson, 2005), which is very large for this species.

### *Sporophyte life span and dormancy*

*B. multifidum* sporophytes are long lived; Stevenson (1975) examined rhizome leaf scars and conservatively estimated large plants to be at least 100 years old, while even small plants were aged to be 15-30 years old. Many species in subgenus *Botrychium* can experience long dormancy periods (Anderson, 2006), which may be linked to the health of its mycorrhizal associates (Anderson, 2006), although the full causes of dormancy are unknown. Although Anderson (2005) did not find evidence of dormancy in his extensive review, a few years later Mesipuu et al. (2009) studied *B. multifidum* populations in Estonia and noted dormancy periods of 1-3 years in a 5-year study; a longer study is needed to assess if dormancy periods may be even longer. Upon reemerging, dormant plants were likely to sprout as non-reproductive plants (Mesipuu et al., 2009).

### *Spore bank*

Researchers have suggested that *Botrychium* species likely form spore banks (Johnson-Groh, 2002), but spore longevity for *Botrychium* remains unknown (Anderson, 2006). Other ferns with non-chlorophyll-bearing spores have an average viability of 2.8 years, but can range up to 48 years in *Asplenium* and 68 years in *Marsilea* (Lloyd and Klekowski, 1970).

### *Spore germination produces a new gametophyte generation*

*Botrychium* spores are homosporous (Rothwell and Karrfalt, 2008) so only one spore is needed to germinate into a gametophyte. The spores of *B. multifidum* require months of darkness to germinate and will not germinate if exposed to light (Gifford and Brandon, 1978). This necessity for darkness is accomplished by the spore descending one to two inches below the soil surface, possibly through cycles of freezing and thawing over the course of the winter (Anderson, 2006). Upon germinating, the spore must form a mycorrhizal association within a few cell divisions in order to survive (Anderson, 2006). The specific environmental conditions necessary for spores to germinate and to establish a viable gametophyte may not occur every year (Peck et al., 1990).

### *Gametophyte development and fungal associates*

Spores germinate underground into non-photosynthetic multicellular gametophytes that require mycorrhizal fungi for nutrition, grow slowly into an adult gametophyte that produces gametes (egg and sperm), and reportedly can live up to five years (Anderson, 2005). Older gametophytes accumulate more than one species of fungal associates (Diagobo, 1983).

### *Gametophyte reproduction produces a new sporophyte generation*

Self-fertilization is common in *B. dissectum* (McCauley et al., 1985) and *B. virginianum* (Soltis and Soltis, 1986) and may be equally as common in *B. multifidum* (Anderson, 2005). Outcrossing is not prohibited, though, as gametes may be dispersed through soil by water or the burrowing movements of soil biota including worms, insects, or small mammals, making genetic recombination possible (Gifford and Brandon, 1978; Wagner et al., 1985).

Questions remain about how often the gametophytes reproduce. Johnson-Groh et al. (2002) studied eight species in subgenus *Botrychium* and found many gametophytes and young subterranean sporophytes. However, after an intensive search for *B. multifidum* gametophytes in the soil at five sites in the Sierras, Stevenson (1975) found no gametophytes and few young plants, concluding that sexual reproduction of *B. multifidum* occurs infrequently. *B. multifidum* and others in subgenus *Sceptridium* may indeed have less gametophyte reproduction, potentially contributing to its rarity.

After gametes successfully reproduce, the new young sporophytes may live underground for several years before producing aboveground structures (Mason and Farrar, 1989); during this time, they entirely rely on mycorrhizae for nutrition (Anderson, 2005).

### **Pollinator Dynamics**

Pollination does not occur. *Botrychium* sporophytes produce homosporous spores, and the gametophytes reproduce underground. See Life History section for details.

### **Seed Dispersal**

Ferns produce spores instead of seeds. Spores are analogous to seeds but are produced from non-flowering plants and are unicellular, thus they don't have stored food resources. *Botrychium* sporangia resemble grape-like clusters, each containing thousands of spores (Anderson, 2006). *B. multifidum* releases its spores individually, not in groups of four as in some other *Botrychium* species (Roe-Anderson and Southworth, 2013).

Spores are passively released and wind dispersed (Boch et al., 2016) – a strategy that may deposit them directly below the parent plant, but they are also capable of long-distance dispersal (Anderson, 2006; Wagner, 1972). Peck et al. (1990) found that most *B. virginianum* spores dispersed within 0.3 meters from the source plant if the height of the sporophore was within the herbaceous layer, and the dispersal pattern showed no effect of wind direction. However, spores released from taller emergent sporophores were primarily dispersed at one to three meters away and in a pattern that showed a strong wind effect (Peck et al., 1990). Once spores land on the ground they may be carried further by surface runoff (Anderson, 2006).

*Botrychium* sporophytes are eaten by mammals and invertebrates; these animals can act as an effective vector for fern dispersal. Fern spores can germinate after being eaten and excreted by slugs, crickets, caterpillars, reindeer (Boch et al., 2016), and voles (Anderson, 2006).

### **Habitat**

Over most of its range, *Botrychium multifidum* tends to grow in exposed grassy sites such as fields, meadows, grasslands, pastures, glades, and savannahs, including in eastern North America (Bertin, 2013; Catling, 2008), western North America (Shaw, 2009; Clausen, 1938), Alaska

(Correll, 1950), Poland (Olejnik et al., 2018), Estonia (Mesipuu et al., 2009), and Japan (Nishida, 1960). However, it can tolerate some shade and is sometimes found in the woods in eastern upstate New York (Morton, 1953), brushy fields and dry open woods in Virginia (Wagner, 1946), beech forests in Japan (Nishida, 1960) and open pine woods in Idaho (Clausen, 1943). In New Jersey, it has been found on abandoned pasture, open woodland, wooded slope, and an open thicket in limestone woodland (New Jersey Natural Heritage Program, 2020).

*Botrychium multifidum* size and fertility is not determined by the age of the plant (Anderson, 2005; Wagner and Wagner, 1983; Williams and Waller, 2015). Instead, environmental factors such as differences in sun exposure and water availability are the likely cause for phenotypic plasticity (Anderson, 2005). In regions that are generally more mesic such as eastern North America, smaller plants tend to be in openings and exposed dry sites, while larger, lax plants tend to be in shady, damp, rich habitats (Anderson, 2005; Wagner and Wagner, 1983). Clausen (1938) noted that across the southern part of its range in North America, it is more likely to grow on wooded hills and rich woods, becoming larger, more lax, and less leathery. Near Ann Arbor in southern Michigan, within a ten yard stretch, Wagner (1962) found *B. multifidum* plants with a wide size variation related to their sun exposure: in the full sun were small *B. multifidum* plants with compact, pale-green blades 25-35 mm long; in the deep shade were large plants with bright green blades 125-140 mm long; and in between, under dappled shade of shrubs, were intermediate sized plants.

In the more arid regions in western North America, *B. multifidum* growing on shady sites tend to be small and non-reproductive, and in open areas the plants tend to be large and fertile (Anderson, 2005). Although its fertility is strongly correlated with open sunny sites (Anderson, 2005; Wagner, 1946), some shade doesn't entirely prevent sporophore production (Anderson, 2005).

As it's quite diminutive, *B. multifidum* may be easily outcompeted for light resources (Anderson, 2005). Most *Botrychium* species in the subgenus *Sceptridium* grow in somewhat disturbed habitats such as grazed grassy slopes, old fields, second-growth woods, roadsides, trailsides, cemeteries, and sites that have been kept open by some disturbance event within the previous ten years (Anderson, 2005). Natural conditions could be enough to keep a site open enough, such as where it's found on serpentine soils or undisturbed wet meadows (Anderson, 2005; US Forest Service, 2020). Otherwise, moderate disturbances that create these open habitats would need to occur regularly to maintain optimal conditions for *B. multifidum*.

As with all pteridophytes, *B. multifidum* requires substrate that is at least somewhat moist; it is found in geothermally influenced meadows in Yellowstone National Park (Anderson, 2006), subalpine wet meadows in the southern Canadian Rocky Mountains in Alberta, Canada (Shaw, 2009), a bog in Quebec (Scudder, 1954), and in Virginia it was found nearby but not in the swamp itself (Wagner, 1946). It is sometimes described as growing on moss (Wagner, 1946; Kruckeberg, 1976), but can also grow in bare soil or leaf litter (Reeves, 1977).

*B. multifidum* has been reported from a wide variety of substrates in terms of soil origin, quality, and pH. It often occurs on sandy soils (Gleason and Cronquist, 1991) but also grows in granitic soils in South Dakota, silty soils in Rocky Mountain National Park, peatlands in southeastern



Alaska, and highly organic soils in Yellowstone National Park (Anderson, 2005). *B. multifidum* is typically found on subacidic or acidic soils that may be high in organic matter, but also tolerates calcareous soils in South Dakota and Wyoming, and serpentine soils in Vermont and Quebec (Anderson, 2005). It can tolerate some soil compaction (Anderson 2005), however, it likely prefers more soil porosity to enable the spores to move down the soil column (Roe-Anderson and Southworth, 2013). *B. multifidum* seems to tolerate a wide variety of soils and substrates; the presence of its mycorrhizal symbionts may be more important to its success than other soil factors.

### *Mycorrhizal fungi*

Mycorrhizal fungi are essential to the establishment and persistence of *Botrychium* species (Anderson, 2005). All of the fern's life stages rely on mycorrhizal interactions. *B. multifidum* gametophytes live entirely underground and don't photosynthesize, so they rely on mycorrhizal fungi to acquire all their resources necessary for growth and development. As the gametophytes age, they are colonized by multiple species of symbiotic fungi (Diagobo, 1983). Sporophyte roots of *B. multifidum* are also heavily infected by mycorrhizae (Anderson, 2006).

### *Genus communities*

*Botrychium* species commonly grow in genus communities (Wagner and Wagner, 1983), defined as the tendency for species of the same genus to have similar habitat preference and therefore to co-exist in the same habitats (Wagner and Wagner, 1983). Field botanists must take care when identifying *Botrychium* as two species can grow right next to each other. Wagner (1946) describes *B. multifidum* and *B. dissectum* growing so close to each other that their leaves appeared to arise from the same root. Ten other *Botrychium* species were listed as growing adjacent to *B. multifidum* in northeastern North America in meadows and disturbed low forests (Wagner and Wagner, 1983; Wagner and Wagner, 1990).

### *Other plants*

*Fragaria vesca* and *F. virginiana* (strawberry) commonly grow with *B. multifidum* in North America and Europe (Anderson, 2005). Strawberry species – along with most species in the Rosaceae family – have strong arbuscular mycorrhizal relationships and may have mycorrhizal fungi in common with *Botrychium* (Anderson, 2005).

### *Herbivores*

Many invertebrates and vertebrate herbivores feed on ferns. Mesipuu et al. (2009) and Sawamura et al. (2009) were concerned that spore-feeding herbivores could reduce fern recruitment, and in fact, invertebrates (likely snails) in Estonia preferentially fed on sporophores of *B. multifidum*, reducing output of spores (Mesipuu et al., 2009). Elk may also selectively graze *B. multifidum* (Anderson, 2005). Fern herbivory is not necessarily detrimental but can negatively affect fern populations if the sporophyte is consumed before the spores are mature. Otherwise, *B. multifidum* seems to be tolerant of grazing (Anderson, 2005).

## **Wetland Indicator Status**

New Jersey includes three wetland regions. In the Atlantic & Gulf Coastal Plain Region, the wetland status for *Botrychium multifidum* is Facultative (FAC), meaning it occurs in wetlands and non-wetlands. In the Eastern Mountains & Piedmont Region and the Northcentral & Northeast Region, its wetland status is Facultative Upland (FACU); meaning it usually occurs in non-wetlands but may sometimes occur in wetlands (USDA, 2020)

## **USDA Plants Code**

BOMU

## **Coefficient of Conservatism**

In New Jersey, the Coefficient of Conservatism rank for *Botrychium multifidum* = 8; it is native with a narrow range of ecological tolerances and is typically associated with a stable community (Walz et al., 2018).

## **Distribution and Range**

*Botrychium multifidum* is a circumpolar species (Wagner and Wagner, 1983). While it primarily occurs in North America and northern, central, and eastern Europe, it also extends into western and southern Siberia, the Himalayas, central Russia, eastern and northwest Asia, and southern Greenland (Anderson, 2005; Flora of North America, 2020; Mesipuu et al., 2009). Its northern limit is in the subarctic of Greenland, Sweden, and Finland (Anderson, 2005). The few populations found at lower latitudes are at relatively higher elevations. The population at Big Meadows in Shenandoah National Park in Virginia is at an altitude of 3,500 ft. (Wagner, 1946), in the Sierra Nevada Mountains in California at approximately 6,000 ft. (Stevenson, 1975), and the southernmost population in North America is in the White Mountains in Arizona at about 8,000 ft. (Reeves, 1977). These southern high elevation populations may be glacial relicts (Fosberg, 1961).

In North America, it is among a group of ferns that Wagner (1972) describes as “bimetroplitan” – they occur equally in the east and west, but are absent from the Great Plains. *B. multifidum* is more common and populations are more continuous in the cool and wet climates of Ontario, Nova Scotia, and British Columbia (Anderson, 2005).

In New Jersey, *B. multifidum* is recorded from seven counties in the Highlands and Ridge & Valley physiographic provinces in northern New Jersey (New Jersey Natural Heritage Program, 2020). Range maps (BONAP; USDA, 2020) show an occurrence in Monmouth county on the coastal plain; however, Clausen (1943) believes this specimen – along with another from the coastal plain in Arkansas – was inaccurately identified and it may be *B. dissectum* var. *obliquum*.

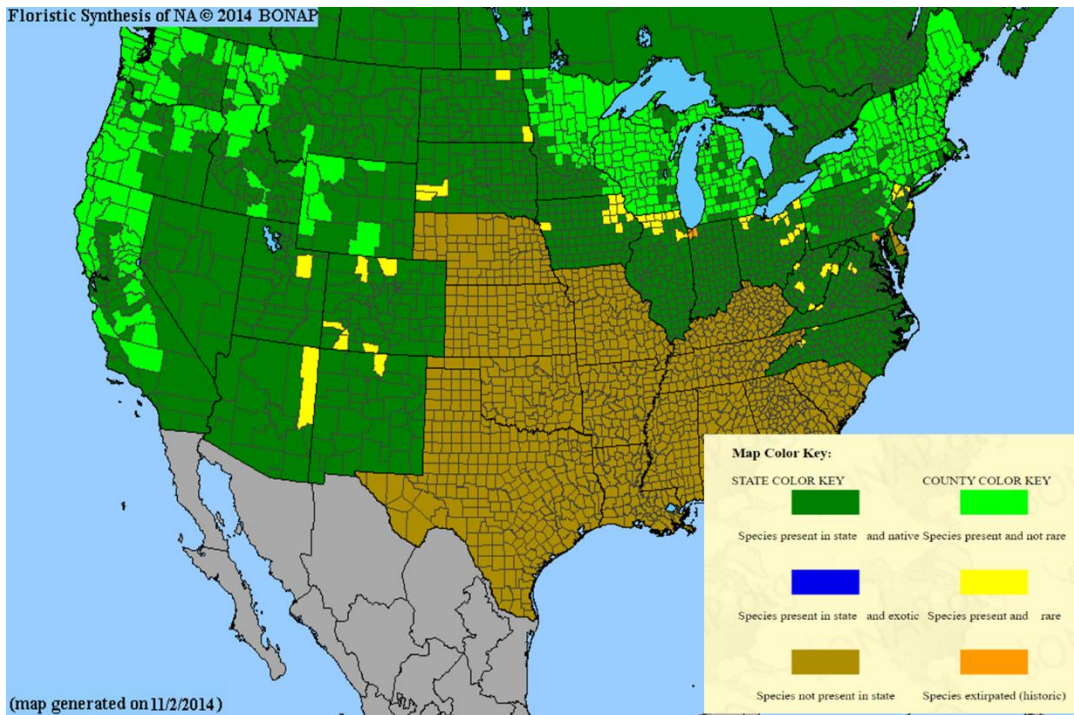


Figure 3. County level distribution of *Botrychium multifidum* in the U.S. (Kartesz, 2015; this website uses the synonym *Sceptridium multifidum*)

### Conservation Status

*Botrychium multifidum* is ranked G5; it is demonstrably secure globally, although it may be quite rare in parts of its range, especially at the periphery (NJ DEP, 2020; NatureServe, 2020).

In New Jersey it is ranked S1; it is critically imperiled in New Jersey because of extreme rarity (5 or fewer occurrences or very few remaining individuals or acres). Elements so ranked are often restricted to very specialized conditions or habitats and/or restricted to an extremely small geographical area of the state. Also included are elements which were formerly more abundant, but because of habitat destruction or some other critical factor of its biology, they have been demonstrably reduced in abundance. In essence, these are elements for which, even with intensive searching, sizable additional occurrences are unlikely to be discovered (NJ DEP, 2020).

New Jersey also ranks *B. multifidum* as Endangered from New Jersey's official Endangered Plant Species List (N.J.A.C. 7:5C – 5.1); it is defined as a species whose survival in the state or nation is in jeopardy (NJ DEP, 2020).

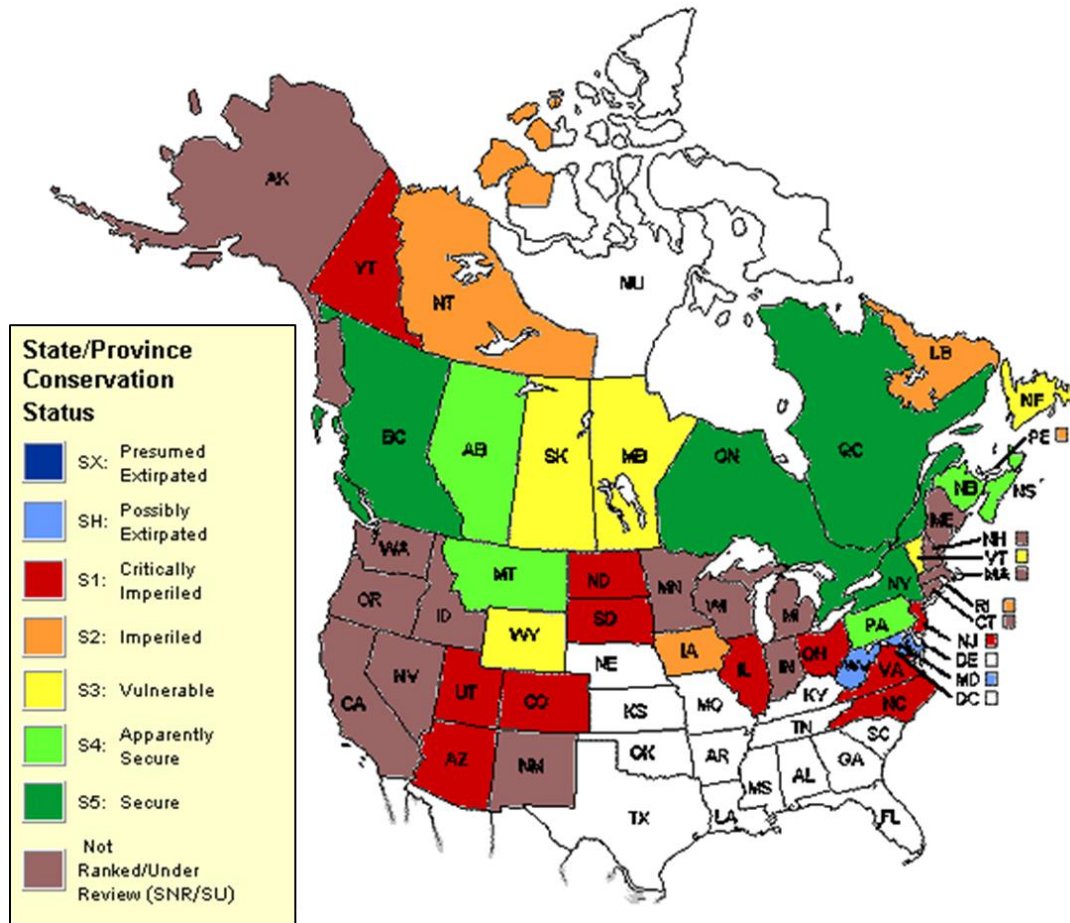


Figure 4. Conservation status of *Botrychium multifidum* in North America (NatureServe, 2020).

## Threats

Ecosystem characteristics most important to *Botrychium multifidum* include canopy coverage, microclimate, hydrology, and mycorrhizal symbionts. Therefore, any changes to these factors on a site that has supported a population of *B. multifidum* over the past decade is a threat to its continued existence (Ahlenlager and Potash, 2007). NatureServe (2020) specifically notes that a lack of disturbance and the consequential successional processes are primary threats to the persistence of *B. multifidum* populations. Additional threats which are applicable to eastern U.S. populations are habitat conversion, recreational activities, global climate change, exotic species invasion, grazing, and the effects of small population size (Anderson, 2005).

## *Succession*

*B. multifidum* in eastern North America prefers open areas within woodlands, woodland edges, fields, and abandoned pasture. In Massachusetts, successional processes were the primary cause of decline as early successional forest matured (Bertin, 2013). Succession to closed canopy forest is a threat, but whether it is due to the changes in light environment, moisture, competition for

other resources, or changes in the mycorrhizal community is unknown (Ahrensleger and Potash, 2007). Under a closed canopy, over-shading reduces sporangia production and reduces spore dispersal (Anderson, 2005).

### *Dormancy*

Although *B. multifidum* produces thousands of spores, there is a markedly small and slow recruitment of new individuals, at least in the above ground sporophyte stage (Mesipuu et al., 2009). Once a sporophyte successfully establishes, it can be very long-lived, but may be dormant for many consecutive years, so monitoring a population is challenging and requires a dedicated commitment to surveying annually. Intermittent surveys are not likely to capture the true population size. Underrepresenting or entirely missing the population could result in missed opportunities for protection and habitat restoration activities.

### *Nonnative invasive plants*

Non-native invasive plants are a threat to *Botrychium* populations. Invasive shrubs and trees such as *Eleagnus umbellata* (autumn olive), *Rosa multiflora* (multiflora rose), *Berberis thunbergii* (Japanese bittersweet), *Rhamnus cathartica* (common buckthorn), *Frangula alnus* (glossy buckthorn), *Acer platanoides* (Norway maple), and *Euonymus alatus* (burning bush) invading an old field or woodland understory are likely to cast too much shade to *B. multifidum*. Additionally, the herbicides used to treat invasive plants can kill *Botrychium* species (Ahrensleger and Potash, 2007) and harm the mycorrhizal community (Druille et al., 2013; Helander et al., 2018).

### *Nonnative invasive earthworms*

Earthworms are a possible threat to *B. multifidum*. Morrison (2017) speculated on the role of earthworms on herbaceous plant communities in forests in New Jersey but their research did not address the issue directly. Earthworms are responsible for negative effects on the rare fern *Botrychium mormo* in Michigan (Gundale, 2002; Zlonis and Henderson, 2018), but there is no research on earthworm effects on other species of *Botrychium* (Anderson, 2005).

Earthworms are directly detrimental to herbaceous plants by feeding on plant roots (Dobson and Blossey, 2015; Hale et al., 2006) and indirectly detrimental by consuming the organic soil horizon (Dobson and Blossey, 2015) and altering the mycorrhizal community that plants depend on (Eisenhauer et al., 2011; Hale et al., 2006; Knowles et al., 2016; McLean et al., 2006; Paudel et al., 2016). Drought and erosion are enhanced on sites missing the organic horizon (Dobson and Blossey, 2015). Nonnative earthworms can also affect pteridophyte reproductive ecology by their interaction with spores, gametophytes, and gametes (Hamilton, 1988; Hamilton and Lloyd, 1991).

Some positive effects of earthworms on ferns have been found. A study of four common ferns in New York found that earthworm presence was positive to some fern species' growth (Bowe et al., 2020). In Ohio, an experiment found positive effects of having earthworms in garden pots with ferns (Hamilton and Lloyd, 1991). Not all plant roots are grazed by earthworms (Dobson

and Blossey, 2015), and their feeding patterns may actually increase arbuscular mycorrhizal fungi (Dempsey et al., 2013) or the fungal spores (McLean et al., 2006). Nonnative earthworm effects on *B. multifidum* are unclear, but see the section *Interacting effects of multiple threats* below.

#### *Herbivory by overpopulated white-tailed deer*

Browsing pressure from over abundant white-tailed deer populations are devastating to native plant communities (Davalos et al., 2015). Deer browsing on *Botrychium* is not mentioned in the literature; it is not known if deer browse *B. multifidum* or avoid it. Plants can avoid deer browsing by being very small (<10 cm) or unpalatable due to chemical defenses or low nutrient quality (Dobson and Blossey, 2015). As a leathery and small statured plant, *B. multifidum* may often escape deer browsing as does *Gaultheria procumbens*, *Mitchella repens*, and *Epigaea repens*. However, *B. multifidum* tends to grow larger in the shade and has the ability to produce spores from small or large individuals, so a tall sporophyte would be in the deer-browsing danger zone. Herbivory of the sporophore before spores are mature would be detrimental to maintaining populations, whereas grazing of the sterile fern frond probably would have little impact because mycorrhizae sustain the plant so well that it doesn't need to entirely rely on photosynthesis for all of its nutrients (Ahrensleger and Potash, 2007).

#### *Interacting effects of multiple threats*

The interacting and compounding effects of a large deer population, invasive plants, and invasive earthworms is having negative consequences for native plants in eastern and midwestern states. In the eastern U.S., overabundant deer herds are charged with being the major driver of decreasing native plant cover; they heavily browse native plant species and generally avoid nonnative plants, thereby reducing competition and indirectly facilitating both nonnative worms and invasive plants (Davalos et al., 2015; Morrison, 2017; Nuzzo et al., 2017). Nonnative earthworms and deer can also interact to stress native plant communities and mycorrhizal communities which, once weakened, allowed for expansion of invasive plants (Nuzzo et al., 2017). This trifecta of stressors exists in the very New Jersey ecosystems where rare native plant species such as *B. multifidum* live.

#### *Climate change*

New Jersey populations of *B. multifidum* are near the species' southern range limit in eastern North America (aside from glacial relics in the Appalachian Mountains). They are considered rear edge populations and may be important sources of genetic diversity (Bertin, 2013), making them highly valuable to the survival of the species as a whole. Unfortunately, the southernmost populations of plant species with more northern distributions will be hard hit by the world's warming climate. Warmer winters will negatively affect northern species that are adapted to periods of cold stratification. For example, *Asplenium scolopendrium* var. *americanum*, a rare fern in New York, is in decline due to physiological stress linked to climate change (Testo and Watkins, 2013). Both stressful hot summers and milder winters will likely threaten New Jersey populations of *B. multifidum* in a similar way.

### *Physical soil disturbance*

Activities that have the potential to crush or uproot plants, disturb the soil, and break mycorrhizal connections threaten *B. multifidum*. Some examples of these activities include off-road vehicle use, trail and road construction or maintenance, timber harvesting, hiking, and camping (Ahlenlager and Potash, 2007; Anderson, 2005). Known populations of *B. multifidum* in New Jersey must be buffered in areas where these activities occur.

### *Small populations*

A common threat to rare organisms is that small populations are less genetically diverse and subject to inbreeding depression, therefore making them more vulnerable to a rapidly changing environment (Mesipuu et al., 2009). *B. multifidum* gametes self-fertilize most of the time and this may have historically benefitted the species by preserving genetic stability and thus maintaining the important relationship with their mycorrhizal fungal symbionts (Anderson, 2005). However, selfing is a strategy that may not serve species as well when the environment changes more rapidly, drastically, and in unexpected ways from those under which it evolved. The small populations of *B. multifidum* in New Jersey may not be genetically equipped to endure the current rapid pace of climate change.

## **Management Summary and Recommendations**

Managing for *B. multifidum* will require a variety of preventative and prescriptive actions. For stable populations, focus should be on maintaining conditions such as the light regime, habitat and microclimate, canopy openness, and hydrology (Ahlenlager and Potash, 2007). Declining populations may need more active restoration, such as tree removal to provide early and middle successional habitats. All activities should be conducted with care to avoid excess soil accumulation, avoid invasive plant establishment, avoid disturbing existing plants and substrate (organic horizon and bryophytes), and minimize impacts to below ground plant structures, fungal hyphae, and mycorrhizae (Ahlenlager and Potash, 2007).

### *Prevent succession to canopy closure*

Maintaining more open habitats, edges, and forest canopy gaps is suggested for *B. multifidum* based on observation but has not been experimentally tested. It's unclear what frequency and intensity of disturbances would most benefit *B. multifidum*, but canopy closure must be prevented. Light to moderate disturbance is recommended for most *Botrychium* species to create and maintain early to mid-successional communities and edge habitat that would have historically been generated through the natural disturbance processes of wildfire and windthrow. Along with maintaining existing populations, land managers should also work to create open habitat near to known populations so that the dispersing spores of *B. multifidum* can establish new populations (Ahlenlager and Potash, 2007). Thinning and burning treatments can both be effective, but specific management decisions will depend on the current conditions of the area. Woody plant removal should be conducted when the ground is frozen to avoid damaging *B. multifidum*'s above and below ground structures (Anderson, 2005).

### *Prescribed fire*

Prescribed fire had positive effects on *B. campestre* in Iowa but has not been tested explicitly for *B. multifidum*. Fire may be beneficial in two key ways: 1) keeping a site open by killing small woody stems to prevent succession to closed canopy, and 2) by exposing mineral soil to promote spore movement underground (Anderson, 2005). Additionally, the contractile roots that keep the bud below the soil surface provides protection from surface fire. Fire applied from late fall through late winter would be least harmful to pre-existing mature sporophytes. The effects of fire to *B. multifidum* would not be visible for some time, as gametophytes take years to mature, and new sporophytes would not be visible above ground until long after the fire event (Anderson, 2005).

### *Reduce white-tailed deer*

Severe reductions in deer density will both protect native plants and stem the expansion of invasive plants. High fencing or cages erected around known populations of *B. multifidum* could prevent deer from browsing tall healthy sporophores, allowing for increased potential for spore maturation, distribution, and germination.

### *Eradicate invasive plants*

Nonnative invasive plants should be removed from sites with *Botrychium multifidum* to prevent over-shading of the ferns. Herbicides can disrupt mycorrhizae (Druille et al., 2013; Helander et al., 2018) so other removal methods are preferred (Anderson 2005). If herbicides are used, direct chemical application to the target plant is preferred over broadcast spraying methods (Anderson, 2005).

### *Prevent recreation and off-road vehicle access*

Blocking all types of recreational access at known *B. multifidum* sites in New Jersey could go a long way towards decreasing negative impacts to our small and struggling populations.

## **Detection and Monitoring**

*Botrychium multifidum* often is very small and can be inconspicuous among dense and taller vegetation, and its cycles of dormancy and the subterranean gametophyte generation contribute to it being a cryptic species. Knowing that it can easily be overlooked, it's important to devote more time and effort into surveying for new populations and monitoring known sites (Anderson, 2005).

*B. multifidum* can be very tiny (smaller than a dime), and young sporophytes look superficially similar to other emerging forbs, so spring is not a good time to look for it. Surveys should be conducted in the late summer and early fall when other species are withering and not as green.



Late in the growing season, *B. multifidum* should be more apparent as it's most likely to have a sporophore which is held vertically and turns yellow as the spores mature (Anderson, 2005).

Botanists must be well-prepared to know how to identify the multiple species of *Botrychium* that could occur in their region. Correctly identifying *B. multifidum* can be difficult. The sub-genera morphological differences are subtle, the size and leaf shape can vary in sun versus shade, it tends to grow together with its congeners in genus communities, and complicating matters further for botanists, often herbarium specimens are misidentified and contain multiple *Botrychium* species on a single sheet (Anderson, 2005; Wagner and Wagner, 1983).

Because detection is an issue in cryptic species, any single survey should be considered an underestimate of the true population size (Anderson, 2005). *B. multifidum* populations need to be monitored and mapped on an annual basis and over an extended period of time in order to gain an accurate estimate of population size and extent. The use of occupancy models that account for imperfect detectability of *B. multifidum* – related to intermittent dormancy and the underground gametophyte life stage – is recommended to improve analyses of monitoring data (Zlonis and Henderson, 2018).

### **Research Needs**

The subterranean gametophyte generation is an important life stage but is poorly understood. It was only by focusing on the gametophytes of the rare fern *Asplenium scolopendrium* var. *americanum* that Testo and Watkins (2013) were able to determine the specific causes of decline. There can be great differences in the selective pressures experienced by the gametophyte and the sporophyte of the same species which cannot be ignored when aiming to successfully conserve rare fern species (Testo and Watkins, 2013).

Mycorrhizal fungi are necessary symbionts to *B. multifidum* in its gametophyte and sporophyte life stages and are important for its establishment and persistence. Important details of their interactions are still unknown such as the specific species of mycorrhizal fungi that colonize *B. multifidum*, how these species differ over the fern life stages, and what environmental factors are important to the persistence of the fungi and its symbiosis with the fern (Anderson, 2006).

Additional research that would contribute to conserving *B. multifidum* includes understanding the required disturbance regime, fluctuations in dormancy versus true mortality, and long-term population viability (Williams and Waller, 2015).

## Synonyms

Many names have been authored for *Botrychium multifidum* as botanists have wrestled with the great variation in size, leaf shape, and leaf margins (Anderson 2005). Table 1 lists the accepted names, basionym, and synonyms for *B. multifidum*. The first accepted name listed (in bold) is used by the New Jersey Department of Environmental Protection.

Scientific Name	Sources	Accepted Name, Basionym, or Synonym
<b><i>Botrychium multifidum</i> (S.G. Gmel.) Trevis.</b>	USDA	Accepted
<i>Botrychium multifidum</i> (S.G. Gmel.) Ruprecht	FNA, ITIS, TROP	Accepted
<i>Osmunda multifida</i> S.G. Gmel.	FNA, TROP	Basionym
<i>Botrychium californicum</i> Underw.	FNA, ITIS, TROP, USDA	Synonym
<i>Botrychium coulteri</i> Underw.	FNA, ITIS, TROP, USDA	Synonym
<i>Botrychium matricariae</i> (Schrank) Spreng.	ITIS, USDA	Synonym
<i>Botrychium multifidum</i> ssp. <i>californicum</i> (Underw.) R.T. Clausen	ITIS, USDA	Synonym
<i>Botrychium multifidum</i> ssp. <i>coulteri</i> (Underw.) R.T. Clausen	ITIS, USDA	Synonym
<i>Botrychium multifidum</i> ssp. <i>silaiifolium</i> (C. Presl) R.T. Clausen	ITIS, USDA	Synonym
<i>Botrychium multifidum</i> var. <i>californicum</i> (Underw.) M. Broun	ITIS, USDA	Synonym
<i>Botrychium multifidum</i> var. <i>coulteri</i> (Underw.) M. Broun	ITIS, USDA	Synonym
<i>Botrychium multifidum</i> var. <i>intermedium</i> (D.C. Eaton) Farw.	ITIS, USDA	Synonym
<i>Botrychium multifidum</i> var. <i>silaiifolium</i> (C. Presl) M. Broun	ITIS, USDA	Synonym
<i>Botrychium silaiifolium</i> C. Presl	FNA, ITIS, TROP, USDA	Synonym
<i>Botrychium silaiifolium</i> var. <i>coulteri</i> (Underw.) Jeps.	ITIS, USDA	Synonym
<i>Sceptridium multifidum</i> (S.G. Gmel.) M. Nishida	TROP	Synonym
<i>Sceptridium multifidum</i> (S.G. Gmel.) M. Nishida ex Tagawa	USDA	Synonym
<i>Sceptridium silaiifolium</i> (C. Presl) Lyon	USDA	Synonym

Table 1. Synonyms for *Botrychium multifidum*. Flora of North America (FNA); Integrated Taxonomic Information System (ITIS); Tropicos (TROP); USDA Plants (USDA).

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