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Survey and Monitoring Protocol for Purple Twayblade (*Liparis liliifolia*)

Prepared through the

Species at Risk Stewardship Program

north-south ENVIRONMENTAL

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Cover Photo: Purple Twayblade (*Liparis liliifolia*) flower by Pauline K. Catling.

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Survey and Monitoring Protocol for Purple Twayblade (*Liparis liliifolia*)

1. Introduction and Objective

The protection of Species at Risk (SAR) and their habitat requires comprehensive and up to-date knowledge of species identification, classification, distribution, occurrence, abundance, habitat and threats. When detailed occurrence data are unavailable, field surveys are necessary to determine if a species is present at a site and ascertain its abundance and threats in order to implement SAR protection. However, many SAR are rare, occur at low densities and may be cryptic, making detection difficult. Furthermore, some plant species can remain non-reproductive for extended periods of time limiting the opportunity to see identifying features, which increases the challenges associated with confirming presence and evaluating the status of the population. This survey protocol has been developed to address the need for reliable, consistent and science-based survey methods in Ontario for Purple Twayblade (*Liparis liliifolia* (Linnaeus) Richard ex Lindley), a vascular plant Species at Risk (SAR), which is listed as Threatened under Ontario's *Endangered Species Act* (ESA), 2007. Development of a standardized survey protocol for this species is identified as a high priority action in the Purple Twayblade government response statement (Ministry of the Environment Conservation and Parks [MECP] 2020).

This document reviews existing information on Purple Twayblade including its identification, distribution, ecology and threats. The survey protocol is based on the best available scientific information at the time of publication, including information in scientific publications, technical reports and consultation with botanical experts and species experts. The survey protocol should be reviewed and, if appropriate, refined should new information become available. This document presents a science-based survey protocol that identifies:

- How to evaluate potential habitat and determine survey locations;
- How to identify Purple Twayblade and differentiate it from similar species
- How to complete a presence/ no detection survey;
- How to complete monitoring;
- How to assess habitat quality and potential threats;
- How to assess site condition; and
- How to record and report data collected.

This document includes two different protocols. The objective of the first protocol (**Section 4.3**) is to describe the methods for detecting presence and prescribes the method that is predicted to maximize detection of Purple Twayblade where it may occur. The objective of the second protocol

(**Section 4.4**) is to provide a standardized method for collecting population abundance and dynamics data over time. This protocol provides a standard method for monitoring known locations of Purple Twayblade.

Determining if there is habitat present under the ESA (general or regulated habitat) or the federal *Species at Risk Act* (SARA), 2002 at a site is a complex process that is not limited to presence/ no detection surveys. For example, even at sites where survey results are negative, general or regulated habitat may still be present based on 1) nearby occurrences of the species (e.g., on an adjacent property), and 2) the manner in which the habitat is defined within a regulation, habitat description or other policy. This document provides a protocol for surveying potential Purple Twayblade sites (as defined here) and monitoring known occurrences; however, it does not include consideration of whether habitat is protected under the ESA or SARA or a delineation of regulated habitat. This protocol should be implemented by field biologists with expertise in botany who have acquired all relevant permits and permission for property access to complete surveys of Purple Twayblade.

2. Species Information

2.1. Taxonomy

Purple Twayblade is a member of the orchid family (Orchidaceae) in the tribe Malaxideae and the genus *Liparis* Richard. This species is taxonomically closest to other members of *Liparis* and the genus *Malaxis* Solereder ex Swartz (Magrath 2002; Mattrick 2004; Canadensys 2021). No infraspecific taxa (subspecies or varieties) have been described for Purple Twayblade (Canadensys 2021).

Latin synonyms include *Leptorchis liliifolia* (Linnaeus) Kuntze, *Malaxis liliifolia* (Linnaeus) Swartz, *Ophrys liliifolia* Linnaeus and *Ophrys trifolia* Walter. English vernacular names include Lily-leaved Twayblade, Brown Wide-lip Orchid, Large Twayblade and Mauve Sleekwort. For the purpose of this report the English nomenclature follows Canadensys (2021) and NHIC (2021).

2.2. Identification

For an illustration of orchid morphology see **Figure 1**. For definitions of botanical terms see the **Glossary**.

Purple Twayblade (**Figure 2**) is a terrestrial perennial herb. This species is distinct in appearance when flowering but may be difficult to see as it grows underneath understorey vegetation (COSEWIC 2010), often in shaded habitats where it is inconspicuous. Plants grow from a bulbous corm to heights between 9 cm and 25 cm (COSEWIC 2010; Leslie 2018). Two alternate, oval to elliptic, glossy, fleshy leaves measuring 4 to 18 cm long and 2 to 8 cm wide occur at the base of the plant. A bright green flowering stalk of five to 33 flowers arises from the centre of the leaves (Magrath 2002; COSEWIC 2010). The inflorescence is 4 to 15 cm with small (2 x 1 mm) floral bracts and slender, 5-7 mm long

pedicels. Irregular flowers are mauve to purple and green in colour with yellow pollinia and anthers (Magrath 2002). The fruit of Purple Twayblade is an ellipsoid capsule with slightly winged veins that is approximately equal in length to the stalk but occasionally is shorter (Leslie 2018).

See **Table 1** for measurements of floral parts of Purple Twayblade as described in Flora of North America (Magrath 2002).

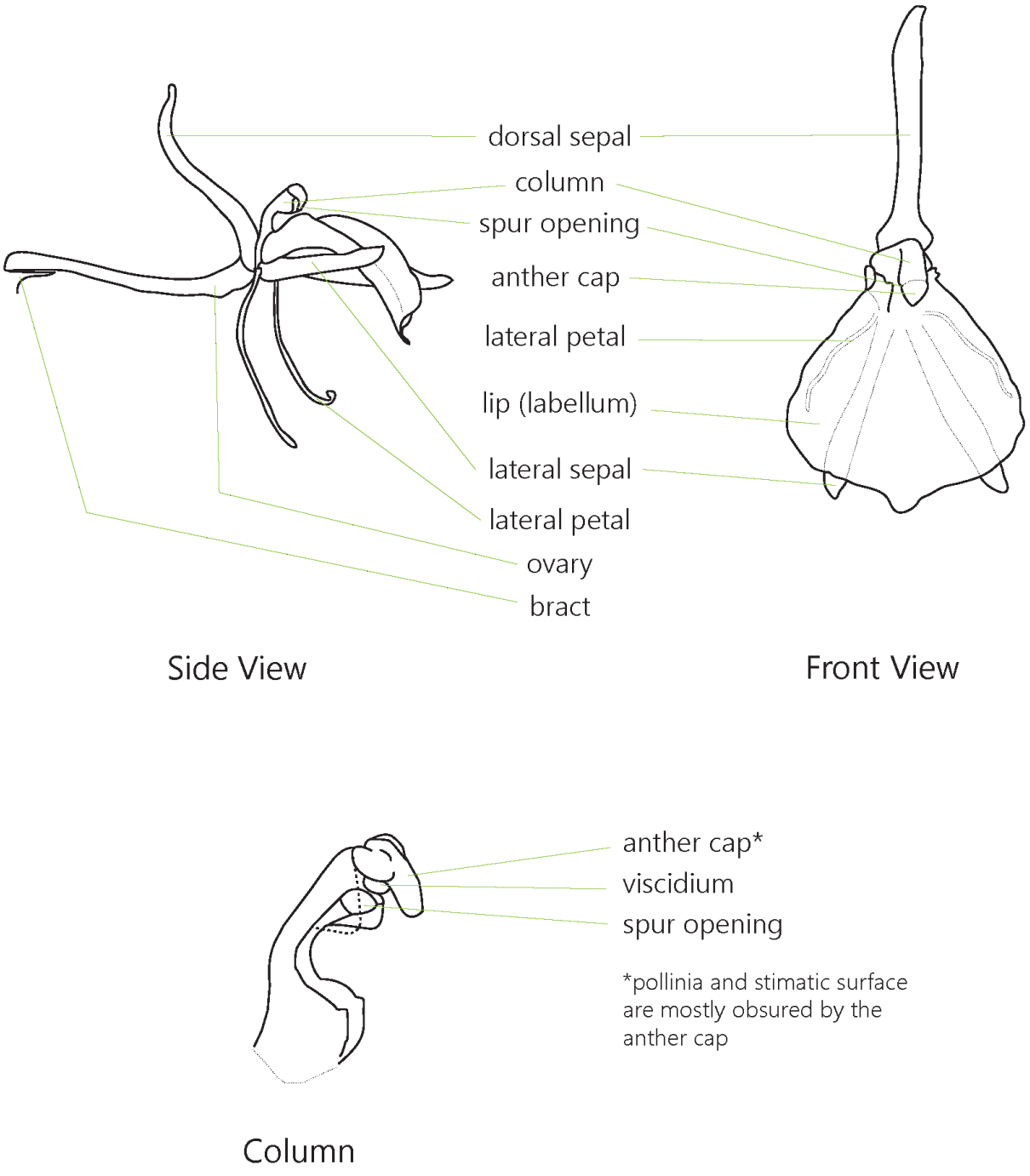

Figure 1. Orchid morphology

Figure 2. Purple Twayblade plant (left) and flower (right) (Photos by P.K. Catling)

Table 1. Characteristics of Purple Twayblade floral parts

Floral part	Shape	Size	Colour
Dorsal sepal	oblong-lanceolate to narrowly lanceolate with an obtuse to acute apex	8–11.5 × 1.2–2 mm	greenish- white
Lateral sepals	oblong-lanceolate to narrowly lanceolate with an obtuse to acute apex	8–11.5 × 1.2–2 mm	greenish- white
Lateral Petals	pendent, curved, tubular, narrowly linear to filiform with strongly revolute margins	8.5–12 × 0.2–0.3 mm	purple to brown
Lip (Labellum)	cuneate-obovate to suborbiculate, base slightly auriculate, apical margin erose-serrulate, apex subtruncate, mucronate	8–12 × 6–10 mm	translucent, mauve to pale purple or rarely green with prominent purplish veining
Column	winged apically with 2 blunt tubercles on inner surface near base	3–4 × 1–1.5 mm	greenish- white
Capsules	ellipsoid with veins often slightly winged	15 × 5 mm	green fading to brown with age

Note: the above table has been summarized from Magrath 2002 and ECCC 2018.

2.2.1. Similar Species

Purple Twayblade is one of two members of the genus *Liparis* that occur in Ontario. Loesel's Twayblade (*Liparis loeselii* (Linnaeus) Richard) also has two leaves at the base and may be mistaken for Purple Twayblade when flowers or capsules are not present. These two species are easily distinguished by flowers and capsules (**Table 2**). Larger specimens of Loesel's Twayblade may be mistaken for Purple Twayblade vegetatively (Mattrick 2004). Loesel's Twayblade is typically lighter green than Purple Twayblade and more likely to occur in wetter habitats.

Members of the genus *Malaxis* in Ontario, which is most closely related to *Liparis*, may overlap in size; however, only Bog Adder's-mouth (*Malaxis paludosa* (Linnaeus) Swartz) has more than one leaf occurring at the base of the plant. Bog Adder's-mouth is smaller than Purple Twayblade and grows in wet acidic swamps and bogs in *Sphagnum* moss hummocks and does not overlap in habitat with Purple Twayblade.

In addition to Loesel's Twayblade and Bog Adder's-mouth, six other species of orchids in Ontario may have two leaves at the base. Species include Showy Orchid (*Galearis spectabilis* (Linnaeus) Rafinesque), Yellow Lady's- slipper (*Cypripedium parviflorum* Salisbury), Pink Lady's-slipper (*Cypripedium acaule* Aiton), Hooker's Rein-orchid (*Platanthera hookeri* (Torrey ex A. Gray) Lindley), Greater Round-leaved Orchid (*Platanthera macrophylla* (Goldie) P.M. Brown) and Round-leaved Rein-

orchid (*Platanthera orbiculata (*Pursh) Lindley). Yellow Lady's-slipper can grow more than two leaves, which if present make those individuals easily distinguishable from Purple Twayblade. Yellow Lady's-slipper and Pink Lady's-slipper have sparsely to densely pubescent leaves while Purple Twayblade have glossy, dark green, hairless leaves. The leaves of Round-leaved Rein-orchid, Greater Round-leaved Orchid and Hooker's Rein-orchid are broader and rounder than Purple Twayblade's, being elliptic to orbiculate or oblate versus the ovate-elliptic shape of Purple Twayblade's leaves.

Showy Orchid is the most similar species to Purple Twayblade vegetatively because these two species both have hairless, glossy green, paired basal leaves that are similar in shape and size. These species can still be distinguished by touching the underside of the leaf. Purple Twayblade and Loesel's Twayblade leaves will be keeled abaxially, whereas Showy Orchid leaves are not (P.M. Catling pers. comm. 2022). It is beneficial for surveyors to be able to distinguish non-flowering individuals as well in case the individual is not flowering in a given year. However, revisiting uncertain individuals in a following year to confirm identification is recommended.

Table 2 notes habitat and vegetative characteristics that may aid surveyors in identification. A visual comparison of some non-flowering plants is provided in **Figure 3**.

Table 2. Comparison of traits between Purple Twayblade and select similar species in Ontario

Common Name (Latin Name)	Leaves	Height (cm)	Flowers	Capsule	General Appearance	Habitat
Purple Twayblade (*Liparis liliifolia*)	Leaves 2, dark green; blade weakly conduplicate basally, dark green, glossy, ovate-elliptic, keeled abaxially, 4–18 × 2–8.5 cm, succulent, apex obtuse to acute	9 to 25	5 to 33, mauve-purple and green; long-peduncled	Pedicel 11–18 mm; body ellipsoid, 15 × 5 mm, veins often slightly winged.	Delicate, slim-stemmed, robust leaves	Mature mesic to moist deciduous forests, pine woods, rich moist humus, often colonizing previously open, disturbed habitats during early and middle stages of reforestation
Loesel's Twayblade (*Liparis loeselii*)	Leaves 2, green; blade weakly conduplicate, green, glossy, oblong-elliptic to elliptic-lanceolate, keeled abaxially, 3.7–18 × 1–4 cm, succulent, apex obtuse to subacute.	6 to 26	2 to 15, green or yellowish green to yellowish white or greenish white	Pedicel 3–7 mm; body obovate to ellipsoid, 9–13 × 3–6 mm, veins often slightly winged.	Bright green, small but robust	Cool, moist ravines, swamps, bogs, or fens, wet peaty or sandy meadows, and exposed sand along edges of lakes, often colonizing previously open and disturbed habitats during early and middle stages of reforestation
Bog Adder's-mouth (*Malaxis paludosa*)	Leaves 2-3, basal; blade elliptic or narrowly elliptic, 0.3–3.5 × 0.1–1.5 cm	3 to 23	2 to 55, green or yellowish green; short-peduncled	Pedicel 2-3mm; body ellipsoid, 4 × 2 mm	Delicate; thin stemmed	Open *Sphagnum* bogs, swampy woods

Common Name (Latin Name)	Leaves	Height (cm)	Flowers	Capsule	General Appearance	Habitat
Showy Orchid (*Galearis spectabilis*)	Leaves normally 2 (occasionally 1), basal, spreading, gradually narrowed to sheathing petiole; blade oblance-ovate to obovate, elliptic, or suborbiculate, apex rounded-obtuse, 9–20 × 2–10 cm	5 to 20	2+; sepals pink-magenta; lip white (rarely pink)	Body ellipsoid, somewhat triangular in cross section	Squat, robust, light green	Moist, calcareous woodlands, thickets, and old fields
Yellow Lady's-slipper (*Cypripedium parviflorum*)	Leaves 2-5, alternate, sparsely to densely pubescent (possibly glabrous when young); blade orbiculate, broadly ovate to elliptic-lanceolate or oblanceolate, 5.2–20.9 × 1.5–14.3 cm	7 to 70	1 to 3, sepals and petals green or yellowish; lip pale to deep yellow	Body ellipsoid to oblong-ellipsoid	Robust, green, multi-stemmed	Mesic to wet fens, prairies, meadows, thickets, mesic to dry open coniferous and mixed forest
Pink Lady's-slipper (*Cypripedium acaule*)	Leaves 2, ascending to spreading, arising directly from rhizome,	15 to 61	1; sepals and petals reddish brown to green; lip	Body ellipsoid to oblong-ellipsoid	Robust, often solitary, dull green, with ribbed leaves	Dry to wet forests, bogs, brushy barrens, heath, and roadsides on highly acidic soil

Common Name (Latin Name)	Leaves	Height (cm)	Flowers	Capsule	General Appearance	Habitat
	pubescent; blade broadly elliptic to oblong-, ovate-, or obovate-elliptic, 9–30 × 2.5–15 cm		magenta to white			
Hooker's Rein-orchid (*Platanthera hookeri*)	Leaves 2, in pair lying on ground; blade orbiculate, broadly elliptic, or obovate, 5–17 × 4–13 cm	18 to 45	2+; flowers resupinate; sepals and petals yellowish green	Body ellipsoid to cylindric	Tall and naked-stemmed; with shiny, flat leaves on the ground	Dry to mesic coniferous and deciduous forest
Greater Round-leaved Orchid (*Platanthera macrophylla*)	Leaves 2, in prostrate pair; blade broadly elliptic, orbiculate, or oblate, 7–24 × 5–19 cm	23 to 63	2+; flowers resupinate, greenish white to white	Body ellipsoid to cylindric	Tall-and naked-stemmed with very large, flat leaves on the ground	Mesic to wet coniferous and deciduous forest
Round-leaved Rein-orchid (*Platanthera orbiculata*)	Leaves 2, in prostrate pair; blade broadly elliptic to orbiculate or oblate, 5–21 × 3–22 cm	17 to 62	2+; flowers resupinate; sepals greenish white; petals white	Body ellipsoid to cylindric	Tall and naked-stemmed, with large, flat leaves on the ground	Mesic to wet coniferous and deciduous forest, fen forest

The above information was summarized from Flora of North America online (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=10638) and from Michigan Flora (https://michiganflora.net/)

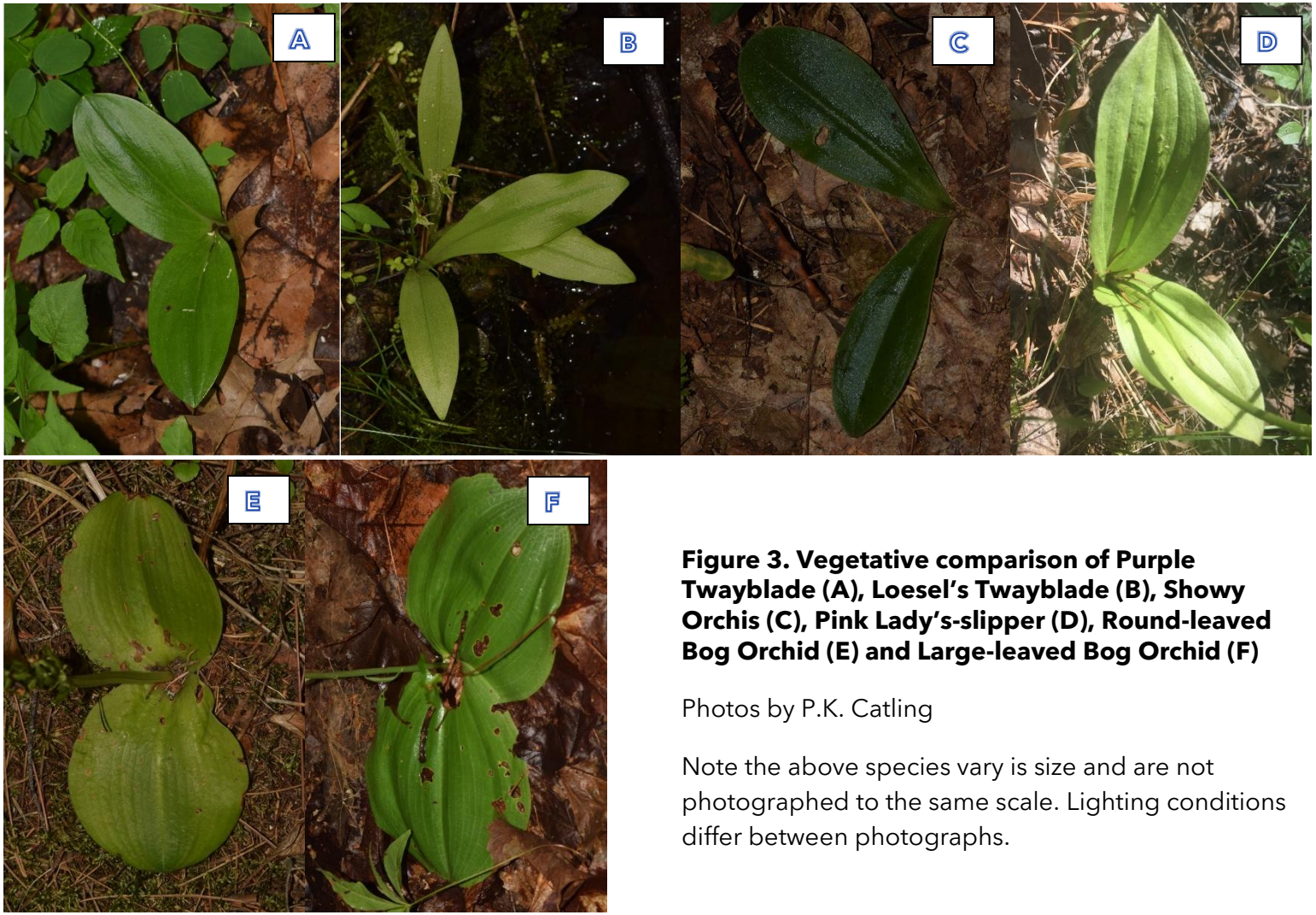

Figure 3. Vegetative comparison of Purple Twayblade (A), Loesel's Twayblade (B), Showy Orchis (C), Pink Lady's-slipper (D), Round-leaved Bog Orchid (E) and Large-leaved Bog Orchid (F)

Photos by P.K. Catling

Note the above species vary is size and are not photographed to the same scale. Lighting conditions differ between photographs.

2.3. Distribution

Purple Twayblade occurs in eastern and mid-western North America (**Figure 4**), predominantly within the Eastern Temperate Forests Ecological Region (EPA 2021). Southernmost occurrences are in northern Georgia and Alabama and northernmost occurrences are in Minnesota, Michigan and Québec. The westernmost occurrences are in Minnesota, Iowa and Arkansas. The easternmost occurrence is in New England (COSEWIC 2010; GBIF 2022). The Canadian and Minnesota populations represent the northern extent of its range.

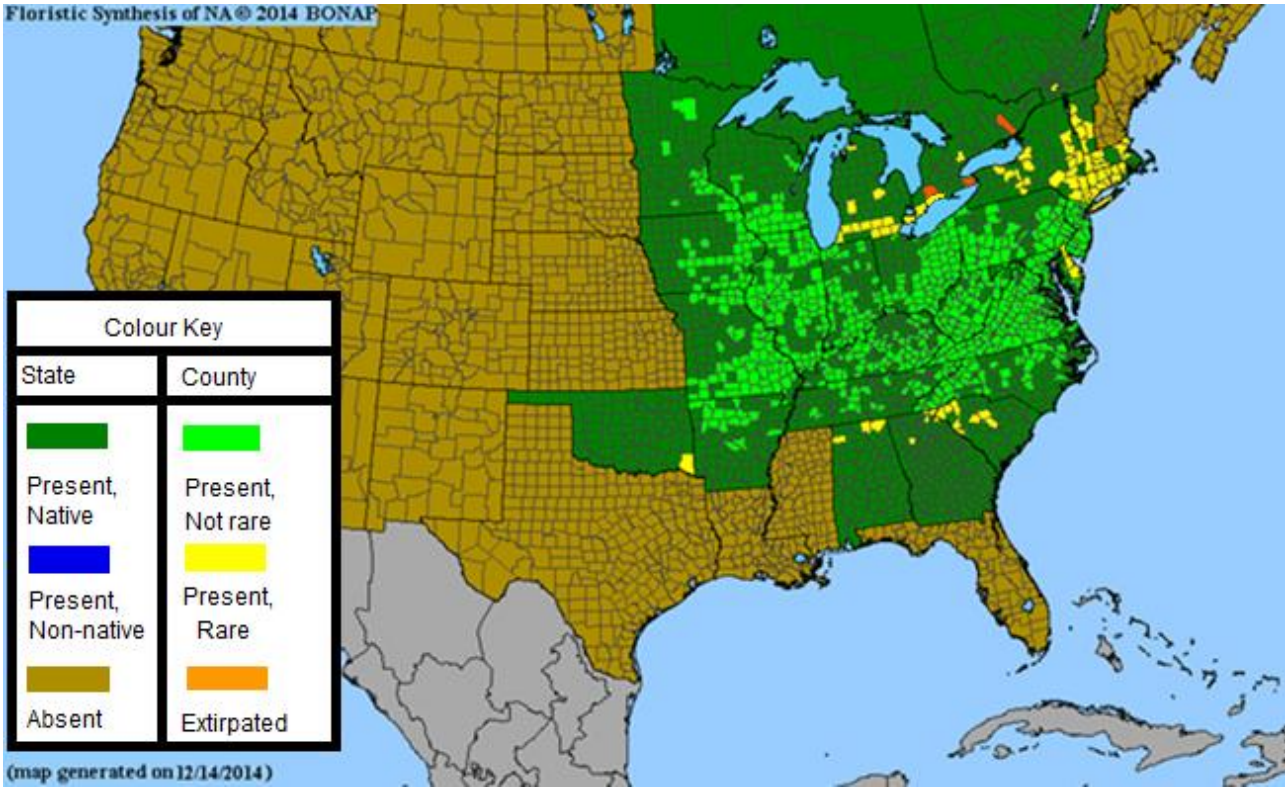

Figure 4. Global distribution of Purple Twayblade. Edited from Kartesz (2015).

In Canada, Purple Twayblade occurs only in southwestern Ontario and southern Québec (**Figure 5**). A total of 26 natural occurrences have been documented in Canada, of which one occurs in Québec and 25 in Ontario (COSEWIC 2010; Catling et. al. 2023; P.K. Catling and W. Van Hemessen pers. obs. 2023). The number of populations known to be extant in Canada in 2008 was nine (COSEWIC 20210). All of the records in Canada occur within the Great Lakes Plains Ecological Area (COSEWIC 2010), including Essex, Chatham-Kent, Middlesex, Elgin, Niagara, York and Frontenac. The Frontenac occurrence may have been extirpated due to drastic habitat change caused by flooding from a Beaver dam (COSEWIC 2010). Based on a range wide inventory of Ontario in 2022 the number of

occurrences known to be extant in Canada is ten (Catling et al. 2023); however, an additional occurrence was located in 2023 (P.K. Catling and W. Van Hemessen pers. obs. 2023).

Table 3 lists the occurrences of Purple Twayblade in Ontario, including occurrences that are considered to be extirpated. Estimates for the number of individuals in Canada ranges from 200 to 3310 plants, with most subpopulations having fewer than 30 individuals (COSEWIC 2010; ECCC 2018). As of 2010, Canadian extent of occurrence was about 41,200 km^2 and the area of occupancy was 19 km^2 (COSEWIC 2010). Unconfirmed or planted occurrences are excluded.

Land ownership includes municipally owned properties managed for conservation within the City of Windsor (Sage Earth Environmental 2019), provincial parks, conservation areas or reserves (owned by NCC or conservation authorities) and privately owned lands.

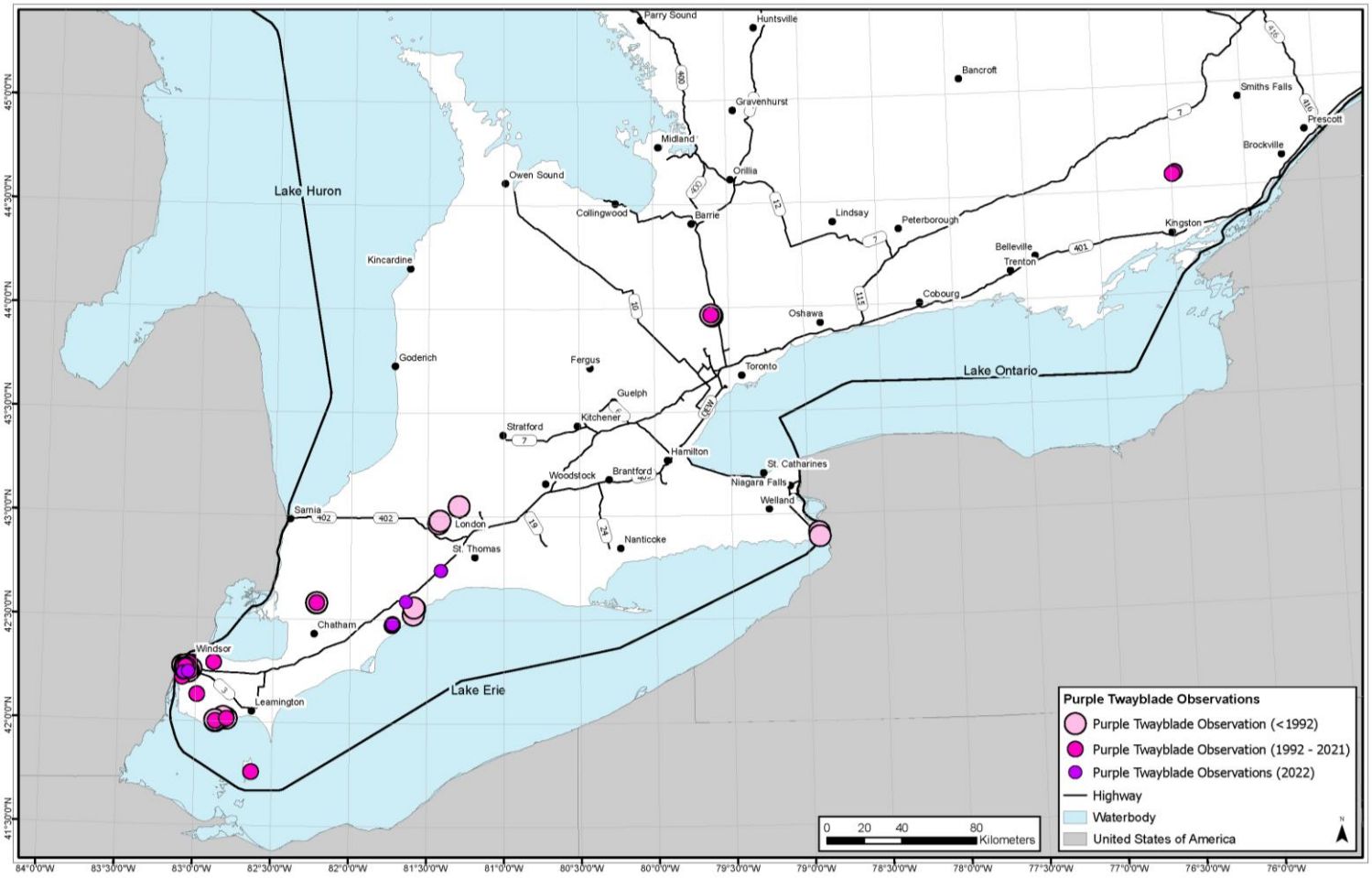

Figure 5. Known occurrences of Purple Twayblade in Ontario, including historical records.

Note that the above map does not include the newly located occurrence in Norfolk.

Table 3. Occurrences of Purple Twayblade in Ontario

Population/ site	County	First Observed	Last Observed	Status	Population Size
Frontenac Provincial Park	Frontenac	2001 (T. Marsh)	2003	Presumed Extirpated	313 total with 187 in flower (2002),400 -500 (2003), 0 (2008); 0 (2022); 0 (2023)
York Region, Happy Valley Forests	York	1977 (R. Tasker)	2000	Unknown	>300 (1977), 34 (1985), 191 (1989), a few (2000), 0 (2001, 2008); *0 (2022)*
Norfolk	Norfolk	2023 (W. Van Hemessen)	2023	Extant	~100 (2023)
Near Arva (London area) *	Middlesex	1940s (J. Higgins)	1950s	Presumed Extirpated	presumed extirpated (2008; 2022) – converted to agriculture
Komoka *	Middlesex	1946 (J. Higgins)	1971	Presumed Extirpated	83 (1962), 4 (1971), 0 (1963), presumed extirpated (2008); *0 (2022)*
Fort Erie *	Niagara	1864 (Day)	1864 (Day)	Presumed Extirpated	presumed extirpated (2008)
Shedden	Elgin	2021 (R. Bramm)	2022 (P.K. Catling)	**Extant**	1+ (2021); 3385 (2022)
Wooded Tract, near West Lorne	Elgin	2020 (P.K. Catling and W.D. van Hemessen)	2022 (P.K. Catling and W.D. van Hemessen)	**Extant**	1 (2020), 0 (2021); 46 (2022)
West Lorne, Allan Craig Woods (near Eagle)	Elgin	1974 (A. Craig)	1985	Historic	24 (1985), 0 (1998); *0 (2022)*
Lakeshore Woods, near New Glasgow	Elgin	1986 (A. Wormington)	1986 (A. Wormington)	Historic	2 (1986), 0 (1998)
Clear Creek	Chatham-Kent	2001 (G. Buck)	2022 (P.K. Catling)	**Extant**	253 (2001), 253 (2002); 33+ (2008); 7+(2011); 1+ (2013); 260+ (2022); 30+ (2023)
Deyo's Woods	Chatham-Kent	1973 (R. Brown)	1997	Unknown	19 (1983), 25 (1984), 10-12 (1997), 0 (2008); 0 (2022)

Population/ site	County	First Observed	Last Observed	Status	Population Size
McAuliffe Woods Conservation Area	Essex	2009 (G. Waldron)	2009 (G. Waldron)	Possibly Extirpated	~40 (2009); 0 (2022)
Windsor (Behind Health Lab)*	Essex	1986 (J. Wilson)	1989	Presumed Extirpated	>70 (1989), presumed extirpated (2008) – converted to manicured parkland
Black Oak Woods ESA (Ojibway Parkway – North)	Essex	1975 (P.M. Catling and S. McKay)	2020 (T. Preney)	**Extant**	>=40 (1975?), 1 (1985), 2 (1990), 29 (2008); 1+ (2020)
Ojibway Prairie Complex (including Tallgrass Heritage Area)	Essex	1975 (P. Pratt)	2021 (A. Woodliffe)	**Extant**	5 (1985), 7 (1989), 23 (2004); 6 (2008); 1+ (2017); 1+ (2021)
Spring Garden ANSI	Essex	1994 (M. Oldham)	2017 (S. Mainguy)	**Extant**	~20 (1994), 4 (2008), 1+ (2011); 116+ (2015); 2 (2017)
LaSalle Woods (Sandwich West Woodlot)	Essex	1979 (J Johnson)	1979 (J Johnson)	Historic	2 (1979), 0 (2008)
Reaume Street Prairie	Essex	1997 (M. Oldham)	2022 (P.K. Catling and G. Pitman)	**Extant**	~40 (1997), 0 (2008); 21 (2022)
LaSalle Woods ESA	Essex	2002 (T. Preney)	2022 (P.K. Catling)	**Extant**	2-4 (2002); 4+ (2018); 53 (2022)
Town of LaSalle Candidate Natural Heritage Area TC5/M1	Essex	2008 (G. Waldron)	2022 (P.K. Catling)	**Extant**	20 (2008); 76 (2022)
Town of LaSalle Candidate Natural Heritage Area CH3-M11	Essex	2008 (G. Waldron)	2008 (G. Waldron)	Possibly Extirpated	14 (2008); 0 (2022)
Canard River, Mitchell Property	Essex	2007 (G. Waldron)	2008 (G. Waldron, K. Oliver, H, Bickerton)	Possibly Extirpated	1 (2008); 0 (2022)
Oxley Poison Sumac Swamp	Essex	1985 (G. Allen and M. Oldham)	1986	Historic/ Possibly Extirpated	4 (1986), 0 (2005, 2006; 2022)

Population/ site	County	First Observed	Last Observed	Status	Population Size
Cedar Creek – North	Essex	1984 (M. Oldham)	1985	Historic	12 (1985), 0 (1998, 2008); *0 (2022)*
Cedar Creek – South	Essex	1973 (W. Botham)	1982	Historic	~12 (1982); *0 (2022)*
Pelee Island- Shaughnessy Cohen Nature Reserve	Essex	2002 (J. Ambrose and G. Waldron)	2008 (J. Ambrose and G. Waldron)	Possibly Extirpated	21 (2002); 27 (2008); 0 (2022)

Status definitions:

- Extant- The species has been observed recently and is known to be extant due to recent (within the last 5 years) confirmation.
- Possibly extirpated- The area with a previous record has been recently searched and the species was not located; however, search effort cannot confirm that the species is not present in other areas nearby.
- Unknown- The species has been recorded within the last thirty years. The area of previous record has not been searched since original observation.
- Historic status- The species has not been recorded in the last 30 years and may be extirpated. Historic sites do not have recent search effort in the exact location of a previous record to suggest extirpation.
- Presumed extirpated- The species has not been recorded in the last 30 years and habitat changes (land use change, succession or other disturbance) and/or recent search effort supports assuming the species is extirpated.

*Note: Four of the above occurrences were assumed to be extirpated in the last status report (COSEWIC 2010). The above information has been summarized from COSEWIC 2010; MECP 2019, personal communications and updated with data collected during field inventory by the authors in 2022. Occurrences at Rt. Hon. Herb Gray Parkway in Windsor (AMEC 2014) have been lumped into the Ojibway Prairie Complex based on proximity.

Note: "0 (2022)" in italics under Population Size column indicates sites where the exact location of previous record could not be surveyed due to property access or was unknown. This data should not be used to indicate extirpation from these sites. In the population size column "1+" indicates that at least one individual of the species was observed, but abundance was not noted for this record.

2.4. Status

Purple Twayblade ranges from Secure (S5) to Critically Imperiled (S1) across its range within North America; however, in half of the states it occurs in it has no status rank (NatureServe 2022). Purple Twayblade occurs in two provinces (S1: Québec, S2S3 Ontario) and 29 states:

- S1: Alabama, Connecticut, New York, Oklahoma, Rhode Island, South Carolina, Vermont
- S2: Delaware, Massachusetts
- S3: Georgia, Iowa, Maryland, Michigan, North Carolina
- S3S4: Illinois, New Jersey
- S4: Kentucky
- S5: Virginia, West Virginia
- No Status Rank: Arkansas, District of Columbia, Indiana, Minnesota, Mississippi, Missouri, Ohio, Pennsylvania, Tennessee, Wisconsin.

In Canada, Purple Twayblade was assessed by COSEWIC as Threatened in 1989, Endangered in 1998 and 2001 and Threatened in 2010 (COSEWIC 2010). It is listed as Threatened on Schedule 1 of the *Species at Risk Act* (MECP 2019). Within Ontario, Purple Twayblade is currently listed as Threatened. Previously the species was assessed as either Threatened (1996, 2001 and 2011) or Endangered (1999, 2004, 2008) in Ontario (MECP 2019). Regional ranks for Purple Twayblade in Ontario are provided in **Table 4**.

Table 4. Status of Purple Twayblade in Ontario

Designation	Range	Rank
G rank - Nature Serve[1]	Global	G5- Secure
N rank – Nature Serve[1]	National	N2N3– Imperiled to Vulnerable
COSEWIC[2]	National	Threatened
SARA- Environment Canada[3]	National	Threatened
ESA – COSSARO[4]	Provincial	Threatened
S Rank[1,5]	Provincial	S2S3– Imperiled to Vulnerable
Carolinian Zone[6]	Regional	R - Rare
Middlesex County[5]	Regional	R - Rare
Chatham-Kent[5]	Regional	R - Rare

[1] NatureServe 2022. https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.149011/Liparis_liliifolia
[2] COSEWIC 2010. https://wildlife-species.canada.ca/species-risk-registry/virtual_sara/files/cosewic/sr_purple_twayblade_0911_eng.pdf
[3] Schedule 1 of the Canadian *Species at Risk Act* https://laws.justice.gc.ca/PDF/S-15.3.pdf
[4] *Ontario's Endangered Species Act*, 2007 https://www.ontario.ca/laws/statute/07e06
[5] Oldham and Brinker. 2009. Rare Vascular Plants of Ontario, 4th Edition.
[6] Oldham 2017. List of the Vascular Plants of Ontario's Carolinian Zone (Ecoregion 7E). Prepared for the Natural Heritage Information Centre, Science and Research Branch, OMNRF. 135pp.

Designation	Range	Rank
Elgin County[5]	Regional	R -Rare
Essex County[5]	Regional	R -Rare
Peel Region[7]	Regional	Rare
Niagara Region[5]	Regional	H -Historic

2.5. Habitat

Habitat information provided in this document is intended to inform surveys for Purple Twayblade. This is not a description of 'Regulated Habitat' for the species as specified in O. Reg. 832/21 enacted under the ESA.

Purple Twayblade is a colonizing species able to survive in a wide variety of habitats and due to its ability to disperse long distances it may appear at sites where it was previously unrecorded and in new habitats (COSEWIC 2010). This species may be locally abundant within a localized area (Buck and Dobbyn 2007).

2.5.1. Edaphic and Hydraulic Conditions

Purple Twayblade exhibits a wide tolerance of soil conditions, colonizing soils including sand, silt, silt loam and clay loam (Sheviak 1974; ECCC 2018). Soils at Clear Creek and in Windsor have been classified as sand and Burford Loam (Bg)/ Granby Sand (Gs), respectively (Buck and Dobbyn 2007; Sage Earth Environmental 2019). Optimally this species prefers mildly acidic soils (pH 4.5 to 6.6), but it can be found in strongly acidic to neutral (Sheviak 1974; Smith 1993; ECCC 2018).

This species prefers mesic drainage conditions but can survive wet or dry conditions (Sheviak 1974; Mattrick 2004; COSEWIC 2010). Most occurrences in Canada are on well-drained slopes or in deciduous or mixed wood swamps (ECCC 2018). Sites with a mosaic of pit and mound formations may have a variety of moisture regimes and provide increased opportunity for this species to disperse into an area suitable for growth (Buck and Dobbyn 2007). The species is considered a facultative upland species that may be found near but not within seeps or drainage features (Mattrick 2004). In Ontario, this species has a coefficient of wetness of 3 (facultative upland) and is not considered a wetland species (MNRF 2014; NHIC 2022). However, the subpopulation at in Frontenac County and Norfolk County were observed in a deciduous swamp growing on moss covered logs (M. Sly pers. comm. 2022; P.K. Catling and W. Van Hemessen pers. obs. 2023), which is atypical habitat for this species.

[7] Credit Valley Conservation (CVC). 2002. Plants of the Credit River Watershed. https://cvc.ca/wp-content/uploads/2011/01/PlantsComplete.pdf

Presence and abundance of specific fungal associates may be more important than soil type, pH and moisture regime (Mattrick 2004). The dominant mycobiont taxon present in Purple Twayblade is *Tulasnella* (Dearnaley 2007; McCormick et al. 2016). It is uncertain how soil pH impacts the fungal associates of Purple Twayblade.

2.5.2. Ecological Classification and Associate Species

Purple Twayblade is found in a wide variety of vegetation communities (see examples in **Figure 6 to Figure 8**) including old fields, shrub thickets, disturbed woodlands, coniferous plantations, cedar stands, rich hardwood forests, moist forest slopes, dry oak forests or woodland, moist floodplain woods, and prairies (Sheviak 1974; Case 1987; Mattrick 2004; COSEWIC 2018; ECCC 2018; Leslie 2018; Pratt 2018). In Ontario, it is known to occur in open oak woodland and savannah, tallgrass prairie, mixed deciduous forest, shrub thicket, shrub alvar, deciduous swamp (mixed or deciduous) and conifer plantations (Allen 1989; White 2001; Buck and Dobbyn 2002; Ambrose et al. 2004; White 2008; ECCC 2018).

Figure 6. Example of Purple Twayblade habitat in thicket community within Essex County, Ontario.

Note: In the above photo Purple Twayblade individuals were temporarily flagged for monitoring.

Figure 7. Example of Purple Twayblade habitat in young transitional *Populus* woodland within Chatham-Kent County, Ontario

Figure 8. Example of Purple Twayblade thicket habitat in Elgin Country, Ontario

The Clear Creek subpopulation occurs within a Fresh-Moist Oak-Maple-Hickory Deciduous Forest Ecosite (FODM9) with a mix of upland and vernal pool wetland areas dominated by Red Maple (*Acer rubrum* Linnaeus) and Shagbark Hickory (*Carya ovata* (Miller) K. Koch) (Buck and Dobbyn 2007).

In Frontenac, Purple Twayblade is found in a deciduous swamp within a depression of an area underlaid by marble bedrock. Red Maple, Silver Maple (*Acer saccharinum* Linnaeus), Freeman's Maple (*Acer x freemanii* E. Murray), Black Ash (*Fraxinus nigra* Marshall) and American Elm (*Ulmus americana* Linnaeus) represent the dominant canopy cover. Shrub cover included Winterberry (*Ilex verticillata* (Linnaeus) A. Gray) and Speckled Alder (*Alnus incana ssp. rugosa* (Du Roi) R.T. Clausen). Ground cover included Sensitive Fern (*Onoclea sensibilis* Linnaeus), Royal Fern (*Osmunda regalis* Linnaeus) and Dwarf Raspberry (*Rubus pubescens* Rafinesque). The area experiences vernal flooding and Purple Twayblade was found growing on mossy logs (White 2002).

2.5.1. Disturbance and Competition

Purple Twayblade is found in vegetation communities with varying canopy cover. It generally prefers areas with open to semi-open canopy cover and has been found in disturbed areas with thinned canopies (COSEWIC 2010; ECCC 2018). It is known to be a colonizing species and can quickly establish large colonies during the early to middle stages of reforestation following a disturbance (Mattrick 2004). There may be a limited window of time where conditions are suitable for this species (Mattrick 2004). If aggressive early-successional species are present these may prevent Purple Twayblade from establishing until those species decline in the middle stages of reforestation. However, Purple Twayblade also declines as overshading decreases the habitat suitability. Abundance and reproductive success have been noted to decline as shade increases at wooded sites (Sheviak 1974; Mattrick 2004; ECCC 2018). Disturbance that maintains open to semi-open conditions may be necessary to maintain suitability. Large populations have been observed to decline to just a few individuals as conditions became less suitable (Sheviak 1974; Mattrick 2004; ECCC 2018).

Purple Twayblade has been observed along anthropogenic trails and White-tailed Deer trails or laydown areas (P.K. Catling pers. obs. 2022), although it cannot be confirmed if Purple Twayblade is associated with these areas due to the disturbance or other factors. Logging and tree fall have also been noted as disturbances that may be associated with Purple Twayblade; however, it is unknown what effect fire has on this species (Mattrick 2004).

2.6. Ecology

2.6.1. Life Cycle and Reproduction

Purple Twayblade is a perennial species with an estimated generation time of 10 to 20 years (COSEWIC 2010). The lifespan of Purple Twayblade is unknown. Plants reach maturity and may flower within four years (Mattrick 2004); however, they may remain unreproductive for up to fifteen years (Rasmussen 1995). Purple Twayblade has two corms (the previous and the current years growth). The older corm may still have the flowering or fruiting stalk attached and provides nutrients to the young corm that will develop into the next above ground plant. Corms exist close together and are attached via a swollen internode along a rhizome (Mattrick 2004). The newer corm functionally replaces the older one in the fall and develops a new bud to form the next corm. This develops the initial stages of roots into the late autumn with active root growth occurring in spring (Mattrick 2004).

Flowering is at its peak in mid-June but may occur between late May and mid-July (Mattrick 2004; COSEWIC 2010). Purple Twayblade flowers produce nectar to incentivise pollinators and the colouration may have evolved as mimicry to attract pollinators (Mattrick 2004). Pollinators must pass an anther column underneath the lip to gather nectar present at the base of the flower (Mohlenbrock 1970; COSEWIC 2010). The primary pollinators of Purple Twayblade and other *Liparis* are flies (Diptera). The species that pollinate it have not yet been determined but Sarcophagidae (flesh flies)

have been observed frequenting Purple Twayblade (Christensen 1994; Mattrick 2004; COSEWIC 2010; ECCC 2018). Additionally flies in the genus Eunoriste (Dark-winged Fungus Gnats, family Sciaridae) have also been observed on Purple Twayblade (D. Daniel pers. obs. 2021). It is assumed that the pollinator is not a specialist dependent on Purple Twayblade (Mattrick 2004). Flies are considered to be poor pollinators and may visit the flower repeatedly without pollinating it (Mattrick 2004; COSEWIC 2010; ECCC 2018). Orford et. al. (2015) suggests that non-syrphid Diptera are more successful pollinators than previously thought; however, this study did not compare genera within the non-syrphid Diptera.

Purple Twayblade is self-incompatible, and cross-pollination is required to produce viable seed, meaning that multiple individuals must occur within the flight distance of pollinating species in order for Purple Twayblade to reproduce sexually (Whigham et al. 2002 cited in Mattrick 2004). Fruit set in Purple Twayblade is reported to be very low (Mattrick 2004). Fruit set measured across six sites in Ontario ranged from 2.38% to 12.54% of flowers producing capsules and 14.29% to 76.92% of flowering individuals producing at least one capsule (Catling et al. 2023). The average number of capsuled produced varied from 0.14 to 1.69 (Catling et al. 2023).

Each capsule contains a large number of small, dust-like seeds (Gleason and Cronquist 1991). The capsules require atmospheric moisture for dehiscence, which occurs in autumn (Mattrick 2004). The small seeds hold very few nutrients and upon germination they must be colonized by mycorrhizal fungi to obtain sufficient nutrients for development until the plant can photosynthesize (McCormick et al. 2006; COSEWIC 2010; ECCC 2018). The relationship between mycorrhizal fungi and Purple Twayblade is symbiotic and the same mycorrhizal fungi are present in protocorms and mature individuals (ECCC 2018). The fungal associate of Purple Twayblade (within the genus *Tulasnella*) is more widely distributed than the plant, suggesting that recruitment is limited by the randomness of seed dispersal and by the patchiness of the fungus' distribution and abundance (McCormick et al 2016; MECP 2019; ECCC 2018). The fungus species *Rhizoctonia monilioides* has also been noted as a mycorrhizal fungal associate of Purple Twayblade (Currah et al. 1997). Determining the presence of fungal associates in the soil requires molecular analysis of deoxyribonucleic acid (DNA) extracted from soil samples (McCormick et al. 2012). The value of these environmental DNA (eDNA) methods as a means of informing surveys for Purple Twayblade has not been determines and therefore these techniques have not been included as part of this protocol.

It is uncertain if Purple Twayblade can persist in a dormant state underground (ECCC 2018). It has been conjectured that it can remain dormant if conditions are unfavourable for above-ground growth; however, this conjecture has not been tested by research on the species (White 2001; Mattrick 2004). Many terrestrial orchids display dormancy and may be absent above ground for period of time, making it difficult to confirm absence (P.M. Catling pers. comm. 2022). Purple Twayblade has been noted to go through rapid changes in abundance from being apparently absent to abundant and vice versus (G. Buck pers. comm. 2022). A study monitoring Purple Twayblade at eleven sites for 29 years

noted the highest observed abundance at 269 individuals but an average abundance of four individuals, representing a 91% decline in abundance and demonstrating the tendency of this species to have irruptive years (Knapp and Wiegand 2014). There is evidence to suggest that the seeds may be able to persist in the seed bank for up to four years, allowing the chance for exposure to a mycorrhizal symbiont (Whigham et al. 2006). Seed germination occurs in the spring following their production and by May small protocorms have formed (Mattrick 2004).

Long term studies are necessary to document changes in orchid demographics over time (Knapp and Wiegand 2014); however, no such studies have been completed on Purple Twayblade in Ontario.

2.6.2. Dispersal

The seeds of Purple Twayblade are extremely small and can be dispersed long distances on air currents (Dressler 1981; Mattrick 2004; COSEWIC 2010). This species may also be dispersed by water, including snowmelt or surficial flow (Mattrick 2004). Dispersal distance may be variable based on habitat conditions and is expected to be less where Purple Twayblade occurs underneath other vegetation and greater in open communities with greater wind speeds (COSEWIC 2010). Actual dispersal distance for this species is unknown. It is expected that the majority of seed is dispersed within close proximity of the parent plant and that long distance dispersal events to areas of suitable habitat where the species may become established occur rarely (COSEWIC 2010).

Purple Twayblade seeds germinate after a period of winter dormancy in the soil and germination requires mycorrhizal fungi to be present in the soil. Seed germination rate has been found to be low in field experiments (Mattrick 2004). Recruitment in orchids may be limited by the obligate dependence on mycorrhizal fungi, depending on the prevalence and distribution of the latter (Diez 2007). It is uncertain how long Purple Twayblade seeds remain viable in the natural seed bank; however, seeds have been noted to remain viable for four years in seed packets without apparently loss of viability (Mattrick 2004; ECCC 2018).

2.6.3. Herbivory

White-tailed Deer (*Odocoileus virginianus*), rabbits (*Sylvilagus* spp.) and Wild Turkey (*Meleagris gallopavo*) have been observed browsing on Purple Twayblade (COSEWIC 2010). Where White-tailed Deer populations are high, browsing is a prominent threat (Brodribb and Oldham 2000). High abundance of White-tailed Deer has been noted at subpopulations in Ontario (Buck and Dobbyn 2007; P.K. Catling pers. obs. 2022). Purple Twayblade was also observed uprooted from the soil along deer trails (P.K. Catling pers. obs. 2022), presumably from trampling disturbance.

No insect herbivores are known. Invasive invertebrates may alter the habitat or impact Purple Twayblade through herbivory (ECCC 2018). Exotic slugs have caused leaf damage to Purple Twayblade near urban areas (G. Waldron, pers. comm. 2008). Damage was confined to the thinner

leaf tissue between the veins (G. Waldron, pers. comm. 2008). Invertebrate damage to the leaves (**Figure 9**) has been observed at two subpopulations in Ontario (P.K. Catling pers. obs. 2022).

Figure 9. Purple Twayblade with leaves damaged by an invertebrate and the flowering stem cut off.

2.7. Threats and Limiting Factors

For a complete description of threats to Purple Twayblade see the status report (COSEWIC 2010) and recovery strategies (ECCC 2018; MECP 2019). Threats include habitat loss or alteration due to urban and agricultural development, succession (which may be a result of disruption to natural disturbance regimes), invasive species (including plants and invertebrates), hydrological change, population fragmentation, chemical pollution (herbicide, fungicide and pesticide) and inbreeding depression (COSEWIC 2010). This species may be naturally limited by pollinator abundance, pollination rate and/or seed germination rate which may contribute to low reproductive success.

Invasive plants may out-compete native species for resources, alter soil conditions or soil stability and alter disturbance regimes such as fire (Brooks et al. 2004). Invasive plants of concern include Garlic Mustard (*Alliaria petiolata* (M. Bieberstein) Cavara & Grande), European Buckthorn (*Rhamnus cathartica* Linnaeus), and Scots Pine (*Pinus sylvestris* Linnaeus). Garlic Mustard may limit native plant growth by interfering with the formation of mycorrhizal associations (Roberts and Anderson 2001).

European Buckthorn has allelopathic effects on soil microorganisms and native plants (Knight et al. 2007; Klionsky et al. 2011). Effects such as these have the potential to affect the presence and abundance of the soil mycorrhizae upon which Purple Twayblade depends.

Purple Twayblade has been observed in areas dominated by non-native species such as European Buckthorn (*Rhamnus cathartica*), Glossy Buckthorn (*Frangula alnus*), Multiflora Rose (*Rosa multilfora* Thunberg), Tartarian Honeysuckle (*Lonicera tatarica* Linnaeus), Japanese Barberry (*Berberis thunbergia* de Candolle) and Red Raspberry (*Rubus idaeus* Linnaeus) (Buck and Dobbyn 2007; Catling et al. 2023). These species, although non-native, the direct negative effects of these species on Purple Twayblade's growth and survival is unknown; however, they still may out compete Purple Twayblade or indirectly contribute to succession making the site less suitable for Purple Twayblade.

Purple Twayblade is naturally limited by low seed set caused its being an obligate out-crosser and its association with poor pollinators (ECCC 2018). Occurrences in Canada are small (less than a few square metres) and widely separated reducing the potential for cross-pollination (ECCC 2018). Population fragmentation and inbreeding depression may be threats further stressed by limiting factors including low pollination and seed set rates.

The distribution of the fungal associate, within the genus *Tulasnella,* is poorly known in Canada and may limit the potential distribution of Purple Twayblade (ECCC 2018). The distribution of *Tulasnella* may be a limiting factor to the distribution and abundance of Purple Twayblade. Earthworms eat leaf litter and organic matter in soil, reducing the duff layer and altering biotic characteristic of soil (including distribution of carbon, nitrogen and other chemicals, root distribution as well as microbe and fungal diversity), which impacts vegetation communities (Muratake 2003; Hale et al. 2006; Addison 2009).

3. Considerations for Implementing the Protocols

3.1. Protocol Refinement

The monitoring protocol here is based on the review of the available literature on Purple Twayblade, consultation with the various experts who contributed advice and knowledge, the authors' own experience monitoring various rare plant species, including Purple Twayblade surveys completed in 2022. An adaptive approach is recommended whereby the field protocol is refined and improved as data are collected, especially during the collection of baseline data. It is recommended that individuals who undertake the field work comment on the protocol and indicate where it was difficult to apply and to make suggestions for improvement. In making refinements, it is essential that the overall objectives of monitoring population size and health, and documenting threats, be adhered to in order to provide sufficient consistency among sites to allow comparison of data and draw conclusions about the status, protection needs and management requirements of the population.

Many terrestrial orchids display dormancy and may be absent above ground for periods of time, making it difficult to confirm absence (P.M. Catling pers. comm. 2022). Research is necessary to determine if how many years of consecutive annual surveys are sufficient to confirm absence of Purple Twayblade, and to determine if the number of years varies with demographic and environmental conditions. Without habitat restoration or disturbance that restores conditions for above ground growth a period of five years, for example, may be insufficient (G. Buck pers. comm. 2022). This protocol should be updated when additional knowledge is available.

3.2. Management and Recovery

Seeds of Purple Twayblade in Ontario have been successfully germinated at the University of Guelph but were unable to survive being transferred to soil (Allen 1989). Transplantation success in Canada is mixed (Allen 1989). Introductions or augmentations are not recommended for Purple Twayblade conservation (Mattrick 2004). To the knowledge of the authors no transplants have been attempted since the development of the Herb Gray Parkway in Windsor.

Habitat management adjacent to extant subpopulations of this colonizing species may be an appropriate strategy to increase population size through increasing suitable habitat area. Habitat management may create new opportunities for the species to establish. Management may include controlled burns, invasive species removal, tree cutting or beaver dam removal (ECCC 2018; B. Chabot pers. comm. 2022; J. Crosthwaite pers. com. 2022; E. Snyder pers. comm. 2022). Knowing the history of disturbance may be an important factor in targeting survey efforts. Fieldwork reports should include a summary of recent management activities at the sites surveyed.

3.3. Habitat and Species Sensitivity

As an upland species, the habitat of Purple Twayblade is not overly sensitive to trampling; however, introduction or spread of invasive species has the potential to alter the habitat and threaten Purple Twayblade. Measures should be taken to reduce the spread of invasive plant seeds including washing or brushing off mud from clothing, boots and field equipment between visiting sites.

This species is small and inconspicuous and may grow under other vegetation, which obscures the view of the plant. The ability for terrestrial orchids to be dormant underground at times means that there is potential for surveyors to trample plants that are not visible. It is recommended that the monitoring protocol detailed below be completed with minimal walking amongst plants to minimize trampling potential. Soil compaction may also negatively impact mycorrhizal fungi or individuals not visible above ground and it is recommended that walking within the habitat be minimized to the greatest extent possible.

Additional Species at Risk flora and fauna may occur in the same habitat as Purple Twayblade. Surveyors should make themselves familiar with these species and minimize potential harm to them.

3.4. Duration and Frequency of Surveys

There is considerable latitude with respect to decisions about the duration and frequency of presence/no-detection surveys since their intensity is not deduced from the estimated detectability of the surveyed species and a stipulated error probability as in the case of presence/absence surveys. Rather, the intensity of such surveys may be decided heuristically and guidance on survey intensity may be considered defeasible if the surveyor's ability to follow it is constrained by practical considerations. The guidance provided here should be interpreted accordingly.

Survey duration is determined by the size of the area to be surveyed, the width of survey transects and the walking velocity of surveyors during the survey. The selection of walking velocity and transect width involve an inescapable element of arbitrariness but they are constrained by very practical considerations. For instance, too wide a transect (say, 500 m) defies human perceptual capacities to view the parts of the transect farthest from its midline even under optimal viewing conditions. Too narrow a transect (say, 0.25 m) is less than the straddle of most human beings while using a walking gait. In practice, transect width is generally between 3 and 5 m, a narrower width corresponding to denser vegetation cover (see **Section 4.4**). A slow walking velocity of 1-2 km per hour with periodic crouching is recommended for most situations in which surveys are conducted for Purple Twayblade. In general, it is recommended that a property between 10 to 15 hectares may take a day to survey depending on the number of surveyors, habitat, density of vegetation, walking speed and amount of potentially suitable habitat present.

Survey frequency depends on the objective to be achieved by undertaking the surveys. Even if this species is temporarily absent, the habitat may remain suitable, or suitability may be restored through habitat management (P.M. Catling pers. comm. 2022). Of course, if presence is confirmed during the initial survey, then abundance and threat monitoring can be substituted for further surveys. However, if Purple Twayblade is not detected, surveys may be conducted periodically to continue to attempt to establish presence. Annual surveys completed over several years are recommended to minimize the possibility that the survey period corresponds to a period of very low abundance or non-seasonal dormancy in the surveyed population. A long-term survey program may be warranted if there is evidence that a site may be inhabited by Purple Twayblade.

At some locations, site restoration may greatly increase detectability as a function of increased abundance, release from dormancy or decreased vegetation cover overall. In these instances, consideration should be given to complete surveys before and after restoration work. Surveys prior to restoration work are necessary to determine the location, if any, of Purple Twayblade to prevent negative impact to the species. However, surveys after may be necessary to update abundance information and determine the effect of restoration on Purple Twayblade.

Research is needed to estimate survey intensity, i.e. both single survey duration and number of repeat surveys, necessary to infer absence from the non-detection of Purple Twayblade under different site

conditions. Accordingly, until further research confirms what is necessary to confirm absence a conservative approach is recommended. Five years of consecutive transects surveys covering the entire property are recommended to confirm absence from a given property. Consecutive years of transect surveys covering the entire site with negative results may be used to increase the probability that Purple Twayblade is absent from the survey area. This approach accounts for uncertainty regarding dormancy period and potential habitat changes. Note that confirming absence at a given time does not mean the site cannot support Purple Twayblade again in the future and the potential for rehabilitation and management should still be considered.

It is recommended that the frequency of monitoring of populations which are confirmed extant depend on the extent of previous monitoring and the demographic stability of the population. If standardized monitoring has not been conducted in a systematic manner previously, annual monitoring is recommended for a sufficient number of years to model the population trajectory in the absence of significant environmental alterations of the site. The frequency of subsequent monitoring depends on the trajectory of the population and the prevalence of threats to the population. More intensive monitoring is warranted if the population is in decline or subject to significant threats. If habitat management is undertaken, monitoring should be designed to allow for its effectiveness to be assessed. It is recommended that monitoring of existing populations occurs at a minimum of every three years.

3.5. Qualifications of Surveyors

Surveyor experience may influence the probability of detection of Purple Twayblade and surveys completed by inexperienced surveyors can lead to inaccurate results. Surveys for Purple Twayblade should be led by individuals who understand the species' biology to assist with focusing search efforts to areas with the highest probability of locating the species (S. Dobbyn pers. comm. 2022). It is vital that one member of each search team be familiar with orchid identification and able to confidently identify the species and distinguish it from species with similar appearance. The surveyors should also have the ability to interpret aerial imagery, navigate, record the survey track, geo-reference observations using a Global Positioning System (GPS) unit, identify associate species and classify vegetation communities.

When flowering, Purple Twayblade is unmistakable, making it possible for the lead surveyor(s) to be assisted by volunteers or staff who are less familiar with the species. However, Purple Twayblade's small inconspicuous nature makes it possible for those without a search image to trample individuals, specifically if they are vegetative or growing under other vegetation (**Figure 10**). If volunteers or less experienced staff are to be utilized, they should be shown Purple Twayblade in the field in a known, easily accessible representative site prior to assisting with surveys and should be accompanied by an experienced professional while on site. The importance of caution should be stressed to all surveyors.

All native orchids may be susceptible to collection and only trusted volunteers should be utilized to minimize this threat and ensure locations of Purple Twayblade in Ontario are not widely shared.

Utilizing more than two surveyors to survey a site is not recommended due to the potential for trampling and difficulty coordinating systematic transects with a large number of people. If a site is large and more than two people are needed to comprehensively survey it, it is recommended that the site be subdivided into sections for small groups to survey to minimize potential for overlap and impacts of trampling.

Figure 10. Purple Twayblade growing under May Apple

Lead surveyors or GIS specialists handling the record data should have completed the Ontario Natural Heritage Information Centre (NHIC) data sensitivity training.

3.6. Authorization

Surveys or monitoring for Purple Twayblade may require an authorization under the ESA if the proposed activities require collection of plants or plant parts, or there is a risk of negative impact to plants or their habitat. The Project Lead should make an inquiry to SAROntario@ontario.ca or contact the responsible biologist in the Ministry of the Environment, Conservation and Parks (MECP) district where the survey is to be completed to determine if a permit is required or if the survey or monitoring can be authorized under the General Regulation to the ESA (i.e. ss. 23.17.2 of O. Reg. 242/08). Any permits required for the collection of Purple Twayblade specimens or potential harm to individuals or damage to habitat should be acquired prior to commencing fieldwork so that these are already in-hand if collection is needed or either harm or damage occurs. Permit applications should be submitted at least five months prior to proposed fieldwork.

Permits under SARA may be required for completing research in National Wildlife areas or other federal lands. Additional permits may be required from Ontario Parks, Parks Canada Agency, Canadian Wildlife Service or Conservation Authorities if surveys or monitoring are to be carried out in provincial parks and conservation reserves, national parks, national wildlife areas or conservation areas, respectively. Municipalities should also be contacted for permission to conduct research in municipally owned parks. Reporting requirements associated with these permits may differ and should be followed accordingly.

Permission to carry out work on private property should be obtained from the property owner or manager prior to accessing the property. Permission, limitations and expectations for work completed on First Nations land should be established in consultation with the band council prior to accessing the property.

4. Standardized Protocol for Survey and Monitoring

4.1. Records Review

A records review should be carried out prior to undertaking field surveys. Existing occurrence records may help to better scope the field survey or, if extensive data are already available for a site, existing records may eliminate the need for a field survey altogether. The absence of occurrence records from an area does not indicate that the species is absent: suitable habitat must be adequately surveyed before concluding that the species is unlikely to be present (see **Section 4.4.2** for details on survey effort required). The following sources can be consulted for information on Purple Twayblade distribution and occurrence records within Ontario:

- NDMNRF Natural Heritage Information Centre (NHIC) www.ontario.ca/nhic; e-mail: nhicrequests@ontario.ca
- Local Conservation Authorities www.conservationontario.ca

- Status reports from the Committee on the Status of Endangered Wildlife in Canada (COSEWIC); available through the Species at Risk Act (SARA) Public Registry www.sararegistry.gc.ca/default.asp
- Other information sources such as, but not limited to species experts, NDMNRF staff, Provincial Park staff, municipal staff, landowners, local naturalists, conservation focused groups including land trusts, Nature Conservancy of Canada, herbaria, friends of provincial park groups, site-related environmental impact or screening reports, published scientific literature and natural history inventories.

Additionally, recent observations on public biological databases such as iNaturalist and the Global Biodiversity Information Facility may not be included in the NHIC database. Inquiring with the observer listed in the database can determine if the record is associated with a previously known occurrence.

4.2. Survey Timing

This species is most obvious and easily identifiable during its flowering period or when capsules are present. The optimal timing for completing surveys is mid-June. Surveys and monitoring for this species may occur throughout the flowering period between late-May and late-June. Exact time may depend on the weather conditions in the previous weeks of spring and the location of the site in Ontario (e.g., southern location may need to be surveyed earlier in the flowering period). Due to the low pollination rate of this species, surveys during seed set period are not recommended unless it is for a specialized study on reproduction. If the survey is completed too early and misses the flowering period, a second survey should be completed between ten and fourteen days later.

Surveys may be completed during a variety of weather conditions, but days of heavy rain, fog or other weather that reduces visibility should be avoided.

4.3. Potential Suitable Habitat Mapping

Potentially Suitable Habitat mapping was conducted for Purple Twayblade by Jenny McCune using the MaxEnt model (J. McCune pers. comm. 2022). The Potentially Suitable Habitat mapping that has been completed may be used to identify areas where additional Purple Twayblade may occur and target them for survey, but this mapping should not be used to infer absence based on a lack of or low suitability. The Potentially Suitable Habitat mapping completed by Jenny McCune was remapped by North-South Environmental Inc. (**Figure 11**). This mapping is on a very broad province-wide scale and surveyors should consider redoing this mapping on a county scale if finer detail is needed. Presence-only data was used with the following environmental predictors for modelling suitable habitat for Purple Twayblade:

- elevation, slope, aspect, soil texture, soil drainage, surficial geology, annual mean temperature, mean temperature of the growing season, isothermality, mean temperature of the wettest quarter, annual precipitation, total precipitation for the growing season, precipitation seasonality, precipitation of the warmest quarter, land use/land cover (SOLRIS), and forest contiguity (e.g. how many of the 81 cells surrounding the focal cell are forested)

The MaxEnt model outputted a 100 x 100 m raster (.ascii file format) with suitability values of 0 – 100, where 100 indicates that 100% of the other cells have less suitable habitat. Values greater than 33.449 were considered suitable habitat for Purple Twayblade. This output was used to inform potential surveying locations for Purple Twayblade based on suitability scores and distance to known NHIC observations:

1 The MaxEnt modelling results were clipped to all natural areas in Ontario, as identified by SOLRIS v3.0, and converted from a raster to a vector dataset. Each polygon in the vector dataset represents a vegetation community.

2 Each polygon was assigned the following attributes:
   - SOLRIS vegetation community type
   - Distance (meters) from known NHIC observations
   - MaxEnt suitability score (0 – 100)
   - Distance from known NHIC observations rank [0 – 10] [reclassification]
   - MaxEnt suitability rank [0 – 10] [reclassification]

3 A preliminary map was created to identify potential surveying locations for Purple Twayblade by assigning each polygon a total suitability rank. All polygons that had a MaxEnt suitability value of between 70 – 100 were automatically assigned the maximum total suitability rank. The remaining polygons were assigned an aggregate score based on their Distance from known NHIC observations rank + MaxEnt suitability rank.

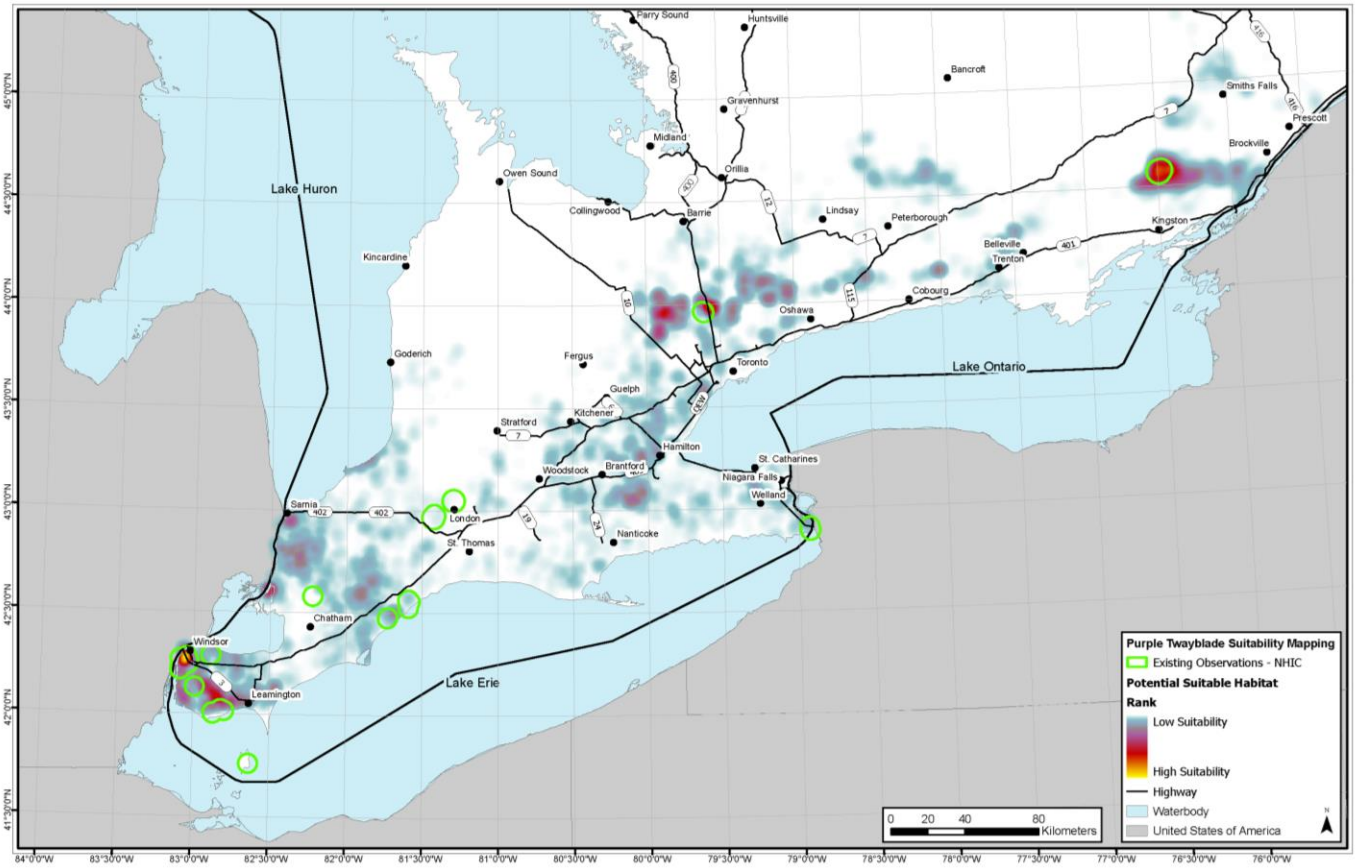

Figure 11. Potentially Suitable Habitat of Purple Twayblade in Ontario (based on data provided by J. McCune).

A review of soil maps for a specific region or site can also help target surveys to more specific locations.

4.4. Presence/ No Detection Surveys

Purple Twayblade may be sparsely distributed or clustered at a site but is typically in small patches within one area (H. Bickerton pers. comm. 2022). The aim of presence/no detection surveys is to determine if Purple Twayblade is present within a given area. This requires that a survey be conducted until Purple Twayblade is detected or until the entire area of suitable habitat has been surveyed without detection. While this protocol is focused on determining presence it is recommended that a methodology to confirm absence should survey the entirety of a property including suboptimal habitats. Certain wetland habitats may be excluded; however, seasonally wet swamp areas may still be suitable. The population in Frontenac County was noted to be growing at the water line on moss covered logs within a swamp (M. Sly pers. comm. 2022). However, since this is the only subpopulation growing in this habitat type it is expected that in southern Ontario swamp habitat may be excluded

from surveys. Surveyors should still consider mosaic communities such as slough swamps, which may include suitable upland pockets interspersed within a larger wetland area.

If the initial presence/no detection survey will inform subsequent monitoring, the entire area of suitable habitat should be surveyed even if Purple Twayblade has been detected. This is expected to maximize the probability of detecting all Purple Twayblade plants or patches of plants that are available to be detected during the survey, thereby supplying the most accurate baseline for succeeding monitoring.

Two methods for presence/ no detection surveys are described. The transect method is the most comprehensive. Targeted search may be utilized if the search is exploratory (e.g., to confirm presence of potential habitats), if the project is not focused on confirming absence or if the budget does not allow for a systematic search of the entire site. Abundance data from targeted search should be considered the minimum abundance present at a site.

Purple Twayblade individuals are easily distinguishable as individuals because they do not reproduce vegetatively, appearing as clumps (genets) with multiple stalks (ramets) that are a single individual plant. In the case of Purple Twayblade each individual will have a single flowering stalk with two leaves at the base. Any vegetative individuals that are unconfirmed should be noted with GPS coordinates recorded for future confirmation.

4.4.1. Transect Method

To maximize the probability of detection, surveyors should walk in parallel transects through all areas with potentially suitable habitat. Transects in areas with little ground cover should be spaced a minimum of 5 m apart. Where resources and timing permits, 3 m apart is recommended. In areas with high amounts of ground cover, which decreases visibility, transects should be spaced 3 m apart. The surveyor should a “crouching” posture periodically throughout the transect survey to detect the smallest and most inconspicuous plants or individuals under other vegetation. A series of transects following a roughly consistent orientation (N-S, E -W) should be walked from one edge of the habitat to the other and then back again to systematically search the entire area; however, extreme care should be used while walking to avoid trampling. Temporary flagging tape or flags may be used to mark transects as they are surveyed and may be particularly useful if multiple people are completing surveys, but these markers should be removed after the survey has been completed. The survey route should be recorded with GPS track log or comparably accurate track log for data collection programs on tablets/ cellphones (e.g., ArcGIS Field Maps, etc.). Multiple surveyors may spread out and complete separate transects at the same time; however, it is recommended they either walk adjacent transects at a similar pace or start at opposite ends of the suitable habitat area and work inward systematically to avoid overlap. Surveyors should scan for Purple Twayblade on either side of the transect. If the species is encountered the area should be searched extensively and patches or individuals should be temporarily flagged to increase visibility and minimize trampling (**Figure 12**).

Figure 12. Example of a patch of Purple Twayblade temporarily flagged with orange survey flags during monitoring and marked permanently with a pigtail stake.

4.4.2. Intuitive Search Method

If the project is not focused on confirming absence and the budget does not allow for a systematic search of the entire site, a more rapid controlled intuitive search can be used. In this method the surveyor walks meandering transects as they see, walk towards and search the most optimal habitats and microhabitats. Data collected from this type of survey can never be used to indicate absence at the site. If Purple Twayblade is located, the estimated abundance should be considered and reported as the minimum number of individual present.

The targeted search method requires that the surveyor be very familiar with orchid identification and have a reliable search image for Purple Twayblade's growth habit and knowledge of its habitat and

microhabitat preferences. Surveyors may walk through the areas of suitable habitat and target the areas that appear most optimal for Purple Twayblade. It is still recommended that this be completed systematically across a site to reduce potential for trampling. Search routes should be recorded by GPS track log or comparably accurate track log on mobile devices. Individuals that have been documented may be temporarily flagged if needed to avoid being counted twice.

4.4.3. Data Collection

After as many Purple Twayblade individuals have been detected in a given area of the site as can be detected given the search effort expended by the surveyors, data should be collected as temporary flags are removed. GPS co-ordinates and photos should be taken of each patch and the number of vegetative and flowering individuals should be recorded. Tally counters (i.e., click counters) are highly recommended for counting larger populations. A blank data sheet has been provided in **Appendix 1**. Surveyors should use this datasheet as a guideline for the minimum amount of data to collect; however, the datasheet may be revised to include additional data for specific survey needs or may be used to develop a digital data collected platform.

If vegetative plants are encountered that are unable to be determined to species, the location should be recorded so that the individual(s) can be revisited in the future. Individuals that have been documented may be temporarily flagged to avoid being recorded twice if necessary.

Survey results, regardless of a positive or negative result, should be reported as outlined in **Section 4.6.**

4.5. Long-term Monitoring

Long-term monitoring (or demographic surveys) tracks individual plant subpopulations. Long-term monitoring is necessary to observe demographic trends and can be particularly challenging to implement for species that exhibit dormancy or rapidly colonize and disappear from sites, as is the case with Purple Twayblade. Species that can rapidly colonize or disappear from sites can experience rapid changes in abundance and require more frequent monitoring to observe population changes that forewarn decline.

Long-term monitoring is more intensive and costly but is necessary to improve our knowledge of the species life history and demographics. Long-term monitoring is also necessary to assess the need for management actions and to determine their success. Data collected can include those required to characterize or estimate population abundance and trends, age or size distribution, recruitment and mortality within different demographics, population health as well as threats and their severity. Initial surveys for the species should follow the presence/ no detection protocol outlined in **Section 4.4**. A systematic transect survey should be completed at the onset of monitoring. A systematic transect survey covering all areas suitable for occupation should be repeated every 10 years to capture individuals that establish elsewhere on the site.

It is recommended that, to the greatest extent possible, all Purple Twayblade individuals within an area be located prior to implementing this protocol so that the best method of permanently marking can be chosen based on their proximity. Temporary markers (toothpicks or thin survey flags) can be placed approximately four centimeters away from the base of each plant to prevent damage to the bulb and root system if needed. Markers should be removed as data is collected for each patch or individual. Additional patches or individuals should be included in monitoring as they are located in subsequent years. A complete search of the site to locate additional individuals should occur every five years and at minimum once every ten years.

4.5.1. Permanently Marking Individuals/ Patches

Permanently marking should only be completed with permission from the landowner/ land manager.

Permanently marking individuals or patches increases the ease of relocating Purple Twayblade and can ensure surveyors are in the right location even if no plants are observed.

Based on the proximity of plants to one another they may be marked individually, or one marker may be used to mark a patch of individuals (**Figure 12**). A patch of individuals should be considered all plants within 1 m of each other. If one marker is used, it should be placed in the middle of the patch if possible. Permanently marking patches minimizes the number of markers placed in the habitat; however, for a demographic study surveyors may wish to mark each individual or record the compass direction and distance from the marker for each individual. If necessary, locations of each individual within the patch can be recorded as distance and compass direction from the central marker (**Figure 13**). At minimum it is recommended that the distance and compass direction to the furthest individuals from the marker be recorded in the notes for the patch.

It is recommended that to mark Purple Twayblade patches either pigtail stakes **(**Error! Reference s ource not found.) with numbered tags and blue flagging tape or metal wire with a numbered tag and flagging tape attached to a nearby tree be utilized. These markers are discreet, thin (minimizing disturbance to the soil or impact to plants), long-lasting and won't be displaced during periods of high water or surficial flow. Markers should be placed a minimum of 4 cm away from any plants to prevent damage to the corm and roots. In locations where collections may be a prevalent threat and public visibility is a concern, either marking without flagging tape or not permanently marking is recommended. People may be drawn to checking out flags, which may lead to plant collection, trampling, or may result in the markers being removed.

Figure 13. Example of method of recording locations of two individuals in a patch of Purple Twayblade

Permanently marking individuals may not be feasible for larger occurrences or in areas that deer frequent. Deer may disturb markers (e.g., tags secured with a nail into the ground) placed on the grounds surface. Permanently marking individuals, if applicable, should use discrete tags to avoid drawing the attention of pedestrians. The exact type of marker for individuals will not be prescribed in this protocol; however, markers should be long-lasting and discrete but relocatable for surveyors who know what to look for. A numbered metal tag secured into the ground with a nail can be used if disturbance by wildlife is not a concern. Nails that trigger metal detectors are recommended for this option since this can be used to relocate where individuals were even if they are no longer present. Plastic markers with numbers engraved may also be used; however, it is recommended that these be thick plastic to ensure they are long-lasting. A monitoring project that used different coloured plastic toothbrushes with numbers engraved noted that the toothbrush markers lasted over 25 years (H.

Bickerton, pers. comm. 2023). The type of marker and tag numbers used must be recorded in fieldwork reports and on the datasheets.

An alternative to permanently marking individuals with tags is photo monitoring. Photo monitoring can provide a useful record of individual presence and locations while minimizing the number of permanent tags and the potential to attract attention. In this case a photo station would be established and marked with flagging tape (distinct from that used to mark the patch itself, if applicable). The compass direction of the photo should be recorded and kept consistent across monitoring years. If multiple photos are needed the compass direction of each should be recorded. Distance to the Purple Twayblade should also be recorded. The surveyor should have a copy of the previous photo with them in the field to aid them in relocating and lining up the photo for consistency. For each photo the Purple Twayblade individuals should be temporarily flagged four inches to the right of their location to avoid damage to the root system. The resulting photo could look like **Figure 12** with bright orange survey flags showing the location of individuals around a single permanent marker.

4.5.2. Data collection

At a minimum surveyors should count and record the number of flowering and non-flowering individuals. The associated plant community should be described, and threats should be evaluated. Example datasheets for monitoring individuals or patches have been provided in **Appendix 2**. If mobile devices are to be used for data collection, it is recommended that the program includes all fields on the datasheet provided.

Notes on individuals in poor health should be taken. Indicators of poor health to note include any evidence of herbivory (browsing or defoliation by insects), decomposition, leaf discolouration (yellow or brown), leaf spotting or evidence of dehydration (weak stem or dry leaves). Invertebrate herbivores should be identified to the greatest level possible or sent to experts for confirmation. Photos should be taken to document the severity of impact.

Demographic monitoring is beyond regular monitoring requirements detailed in this protocol. Data collected for demographic monitoring may include data on height (from the ground to the top of flowering stem if flowering or from the ground to the highest point of a leaf), leaf length (from base to apex), leaf width and number of flowers may be collected to gain a better understanding of population demographics. Demographic monitoring has been excluded from the example datasheet in **Appendix 2** and is considered to be above the requirements for regular monitoring.

4.5.2.1. Associated Plant Community

The Ecological Land Classification system for southern Ontario's vegetation community description framework should be used to describe the associated plant community (Lee et al. 1998 or updated equivalent). For each community in which Purple Twayblade is present, the ELC community boundary

should be mapped, and the dominant species and cover of each vegetation layer should be recorded.

Species considered invasive in Ontario based on Weediness Index[8], exotic status (SE5) in the NHIC Database[9] or other invasive species list[10], should be noted. The abundance of all invasive species in the area of suitable habitat should be estimated (1 = 1-2 plants, 2 = 3-5, 3 = 6-20, 4 = 21-50, 5 = 51-100, 6 = 100+) and distribution described (L=localized, O=occasional, P=scattered patches, W=widespread). Proximity to Purple Twayblade should be noted. For species that may pose a threat, GPS coordinates for the closest individual to Purple Twayblade should be recorded. Polygons of larger patches of invasive species may be delineated.

4.5.3. Evaluating Threats

All threats to the habitat and species in and adjacent to the area of occurrence should be noted and ranked according to the COSEWIC guidelines for evaluating threats (COSEWIC 2012). Relevant pages of the COSEWIC guidelines have been included below. Where possible, the location and extent of threats should be mapped using a GPS or tablet. Where threats are not mappable (e.g., changes in hydrology, widespread distribution of an invasive plant species, evidence of widespread herbivory), they should be described. Adjacent land-uses should also be described.

4.5.3.1. COSEWIC Threat Evaluation

The text from this section was taken directly from page 9-12 of the COSEWIC guidelines for threats classification (COSEWIC 2012). Table numbers have been altered to fit this document.

Scope of a Threat

Scope is defined herein as the proportion of the species or ecosystem that can reasonably be expected to be affected by the threat within 10 years with continuation of current circumstances and trends (**Table 5**). Current circumstances and trends include both existing as well as potential new threats. The 10-year timeframe can be extended for some longer-term threats, such as global warming, that need to be addressed today. For species, scope is measured as the proportion of the species' population in the area of interest affected by the threat. For ecosystems, scope is measured as the proportion of the occupied area of interest affected by the threat. If a species or ecosystem is evenly distributed, then the proportion of the population or area affected is equivalent to the

[8] Oldham et al. 1995. Floristic Quality Assessment System for Southern Ontario. Natural Heritage Information Centre, Ontario Ministry of Natural resources. Peterborough, ON. 17pp.
[9] NHIC Database Available at: https://www.ontario.ca/page/get-natural-heritage-information
[10] Such as those developed by conservation authorities: CVC Invasive Species Lists and Factsheets https://cvc.ca/wp-content/uploads/2012/09/cvc-appendix-landowners-guide-to-invasives.pdf

proportion of the range extent affected by the threat; however, if the population or area is patchily distributed, then the proportion differs from that of range extent.

Table 5. Scoring the scope of identified threats. Typically assessed within a 10-year timeframe.

Scope of threats scoring	
Pervasive	Affects all or most (71–100%) of the individuals
Large	Affects much (31–70%) of the individuals
Restricted	Affects some (11–30%) of the individuals
Small	Affects a small (1–10%) proportion of the individuals
Negligible	Affects a negligible (< 1%) proportion of the individuals

Severity of a Threat

Within the scope of the threat, severity is the level of damage to the species or ecosystem from the threat that can reasonably be expected with continuation of current circumstances and trends (including potential new threats) (**Table 6**). Note that severity of threats is assessed within a 10-year or three-generation timeframe, whichever is longer (up to 100 years).

For species, severity is usually measured as the degree of reduction of the species' population. Surrogates for adult population size (e.g., area) should be used with caution, as occupied areas, for example, will have uneven habitat suitability and uneven population density. For ecosystems, severity is typically measured as the degree of degradation or decline in integrity (of one or more key characteristics).

Table 6. Scoring the severity of a threat (within a 10-year or three-generation timeframe, whichever is longer [up to 100 years]).

Severity of threats scoring	
Extreme	Within the scope, the threat is likely to destroy or eliminate the occurrences of an ecological community, system, or species, or reduce the species population by 71–100%
Serious	Within the scope, the threat is likely to seriously degrade/reduce the affected occurrences or habitat or, for species, to reduce the species population by 31–70%
Moderate	Within the scope, the threat is likely to moderately degrade/reduce the affected occurrences or habitat or, for species, to reduce the species population by 11–30%
Slight	Within the scope, the threat is likely to only slightly degrade/reduce the affected occurrences or habitat or, for species, to reduce the species population by 1–10%

Severity of threats scoring	
Negligible	Within the scope, the threat is likely to negligibly degrade/reduce the affected occurrences or habitat or, for species, to reduce the species population by < 1%.
Neutral or Potential Benefit*	Within the scope, the "threat" is likely to improve or not affect occurrences or habitat or, for species, to be neutral or to improve (a net benefit) the species population by > 0%).

*Threat may have some localized negative effects, but overall is thought to not affect or be a benefit to the species. For example, a forest fire may directly affect some individuals of a browsing ungulate, and produce a short term loss of habitat, however, over the three generation time window there is a benefit to the population as a whole due to regeneration of browse species post fire.

Impact of a Threat

Threat impact (or magnitude) is the degree to which a species or ecosystem is observed, inferred, or suspected to be directly or indirectly threatened in the area of interest. The impact of a threat is based on the interaction between assigned scope and severity values, and includes categories of very high, high, medium, and low.

Threat impact reflects a reduction of a species population or decline/degradation of the area of an ecosystem. As shown in **Table 7**, the median rate of population reduction or area decline for each combination of scope and severity corresponds to the following classes of threat impact: very high (75% declines), high (40%), medium (15%), and low (3%).

Table 7. The relationship of threat impact and population reduction or ecosystem decline or degradation

		Scope (%)			
		Pervasive	**Large**	**Restricted**	**Small**
Severity (%)	**Extreme**	50–100	22–70	8–30	1–10
	Serious	22–70	10–49	3–21	1–7
	Moderate	8–30	3–21	1–9	0.1–3
	Slight	1–10	0–7	1–3	< 1

Very High; High; Medium; Low

It is not always possible to assign an impact category of very high, high, medium, or low to a threat. For a complete list of impact categories, see **Table 8**. These additional categories include:

- Negligible: when the value for scope or severity is negligible.

- Unknown: used when impact cannot be determined (e.g., if values for either scope or severity are unknown).
- Not a Threat: when severity is scored as neutral or a potential benefit.
- Not Calculated: impact is not calculated if threat is outside the assessment timeframe (e.g., timing is insignificant/negligible or low, as threat is only considered to be in the past).

Table 8. Using scope and severity to derive the impact of a threat

		Pervasive	Large	Restricted	Small	Negligible	Unknown
Severity	**Extreme**	Very high	High	Medium	Low	Negligible	Unknown
	Serious	High	High	Medium	Low	Negligible	Unknown
	Moderate	Medium	Medium	Low	Low	Negligible	Unknown
	Slight	Low	Low	Low	Low	Negligible	Unknown
	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Unknown
	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
	Neutral or Potential Benefit	Not a threat	Not a threat	Not a threat	Not a threat	Not a threat	Unknown

Very High; High; Medium; Low

Timing of a Threat

Although timing (immediacy) is recorded for threats, it is not used in the calculation of threat impact. However, threat impact is not calculated for threats where timing values are low or negligible. See **Table 9** for guidance on determining the timing of the threat.

Table 9. Scoring the timing of a threat.

	Timing of threats scoring
High	Continuing
Moderate	Only in the future (could happen in the short term [< 10 years or three generations]), or now suspended (could come back in the short term)
Low	Only in the future (could happen in the long term), or now suspended (could come back in the long term)
Insignificant/Negligible	Only in the past and unlikely to return, or no direct effect but limiting

4.5.4. Documentation and Reporting

The following should be recorded and reported for each occurrence of Purple Twayblade:

- time and date of observation;
- name and contact information of observer(s);
- location description (including coordinates and map datum) and directions;
- area of occupancy polygon and/or coordinates of centroid;
- map of distribution of plants within area of occupancy;
- photo records of occurrence and habitat;
- count or estimate of individuals (reproductive and non-reproductive);
- vegetation community description according to Lee et al. (1998);
- description and locations of nearby invasive species; and
- a description of local threats.

The following should be recorded and reported for each site:

- what method or markers were used to mark locations of individuals;
- if transects were used, their location and dimensions;
- locations (GPS or transect references) and tag numbers of all permanently marked plants;
- an assessment of site wide threats.

This protocol is science-based and has been revised after an inventory of Purple Twayblade in Ontario. It is highly recommended that any issues with the survey method be recorded and reported so the protocol can be improved and adapted in the future.

SAR data should be reported to NHIC.[11] NHIC is Ontario's conservation data centre and maintains records of Ontario's SAR occurrences. Negative survey results should also be submitted to NHIC. Data should be submitted in digital format (spreadsheet or shape files with associated tabular data) as per the instructions on NHIC's website.[12] Incidental observations of other SAR or other provincially tracked species encountered during surveys should also be reported to NHIC, either in digital format or iNaturalist (by joining the NHIC Rare species of Ontario project). A fieldwork summary report should also be submitted to NHIC with mapping and GIS files.

If survey work is completed within a provincial park or conservation reserve, reporting requirements will be defined in the authorization to conduct the work. Reporting requirements or expectations for work completed on First Nations land should be established in consultation with the band council and

[11] www.ontario.ca/nhic
[12] https://www.ontario.ca/page/report-rare-species-animals-and-plants

any protocols for data transfer and use of data are to be followed. Distribution of data collected from First Nations lands is at the discretion of the band.

4.6. Reporting

Datasheets for consistent surveying have been provided in **Appendix 1** (presence/ no-detection survey) and **Appendix 2** (long-term monitoring survey). Scanned datasheets should be included in fieldwork reports unless the reports are to be made public documents. If reports are to be made public documents no specific location data should be included.

All data should be shared with NHIC (https://www.ontario.ca/page/natural-heritage-information-centre). The NHIC is Ontario's conservation data centre and maintains the provincial record of Ontario's rare animals, plants and plant communities. Information regarding the non-detection of Purple Twayblade at a site may also be important and should also be submitted to the NHIC. Data should be submitted in digital format (e.g., spreadsheet, shape files with associated tabular data) as per the instructions on NHIC's website. The local OMNRF/OMECP office should also be provided with a copy of the data submitted to NHIC.

Additional reporting required by MECP, NCC, conservation authorities and/or municipalities should follow requirements of these individual permits. Landowners or managers should be made aware of Purple Twayblade on their property to promote its preservation.

Reporting should include:

- scans of datasheets or digital data files if collected via tablet,
- surveyor names and contact information of the Project Lead,
- a summary of surveyor(s) experience with Purple Twayblade,
- a map of the sites surveyed showing the location of suitable habitat, survey route and location of any Purple Twayblade individuals or unidentified vegetative individuals,
- shapefiles or other digital data associated with mapping,
- survey effort (duration and area covered),
- photographic vouchers of each occurrence including vital identification features and habitat photos,
- site description and general site photos,
- general description of threats, anthropogenic impacts or other factors that might influence absence/ extirpation from the site, and
- if the search result is negative, recommendations for future surveys including areas where future surveys should be focused. Details on potential unconfirmed Purple Twayblade (vegetative and unable to ID) should be provided if necessary.

4.6.1. Voucher Specimens

Conservation of SAR plants should be of primary concern when considering collecting; however, it is also important to ensure that reports of previously undocumented populations of a plant species are supported by sufficient evidence, such as voucher specimens or photo records, and confirmed by experts. Permits under the ESA are required for the collection of Purple Twayblade and the Project Lead must acquire the appropriate permits for collection prior to collecting any plant materials from this species (including eDNA work that may only require a portion of the individual). Voucher specimens should only be taken if recommended or requested by MECP/ NHIC. Voucher specimens should not be taken if identification is in doubt, and it is recommended that any unconfirmed vegetative individuals be revisited rather than collected. As an alternative to voucher specimen collection, photos that clearly document all the identification features may be submitted to NHIC or herbaria.

If collected, voucher specimens should be submitted to an herbarium with the following information provided:

- collector name,
- identifier name,
- collection date,
- location in GPS coordinates,
- location description,
- details on abundance, and
- a general habitat description including associate species.

Material collected from different occurrences should be kept separate and submitted as separate collections. A recommended datasheet to fill out for collecting voucher specimens is provided in **Appendix 3**. This should be considered a baseline for the minimum data collected and may be modified to suit digital formats or for the collection of additional data.

5. Glossary

Abaxial – Referring to the lower surface of a leaf, petal or other lateral organ. Contrast *adaxial*.

Adaxial- Referring to the upper surface of a leaf, petal or other lateral organ.

Acute – Side or margins converging to a less 90˚ angle.

Alternate – Arranged singly at nodes, such as leaves, flowers or inflorescences. Neither opposite or whorled.

Anther – The pollen bearing part of the stamen.

Apex – The tip; the part furthest from the point of attachment.

Apical – At or on the apex of a structure.

Apiculate – Ending at a short and pointed tip.

Auriculate – Ear-shaped lobe or appendage often projecting from the base or summit of an organ, such as a leaf blade.

Bracts – A specialized leaf often positioned under the flower or inflorescence.

Capsule – A type of fruit that dehisces (opens) along two or more sutures, usually several- or many-seeded.

Column – The fusion of stamens and pistil in the Orchidaceae family.

Committee on the Stats of Endangered Wildlife in Canada (COSEWIC): The committee established under section 14 of the *Species at Risk Act* that is responsible for assessing and classifying species at risk in Canada.

Committee on the Status of Species at Risk in Ontario (COSSARO): The committee established under section 3 of the *Endangered Species Act*, 2007 that is responsible for assessing and classifying species at risk in Ontario.

Conduplicate – Leaf that is folded lengthwise with the upper (adaxial) surface within.

Conservation Ranks- Conservation ranks are designations assigned by NatureServe or local scientists to define how rare a species or ecological community is on the global, national, provincial and local levels. Ranks are determined by NatureServe (NatureServe 2022) and, in the case of Ontario's S-rank, by Ontario's Natural Heritage Information Centre (NHIC 2021). The conservation status of a species or ecosystem is designated by a number from 1 to 5 or the letter(s) X, H or NR, preceded by the letter G,

N or S reflecting the appropriate geographic scale of the assessment. The numbers mean the following:

- X Presumed Extinct (species) – Not located despite intensive searches and virtually no likelihood of rediscovery. Presumed Eliminated (ecosystems, i.e., ecological communities and systems) – Eliminated throughout its range, due to loss of key dominant and characteristic taxa and/or elimination of the sites and ecological processes on which the type depends.
- H Possibly Extinct (species) or Possibly Eliminated (ecosystems) – Known from only historical occurrences but still some hope of rediscovery. Examples of evidence include (1) that a species has not been documented in approximately 20-40 years despite some searching and/or some evidence of significant habitat loss or degradation; (2) that a species or ecosystem has been searched for unsuccessfully, but not thoroughly enough to presume that it is extinct or eliminated throughout its range.
- 1 Critically Imperiled – At very high risk of extinction or elimination due to very restricted range, very few populations or occurrences, very steep declines, very severe threats, or other factors.
- 2 Imperiled – At high risk of extinction or elimination due to restricted range, few populations or occurrences, steep declines, severe threats, or other factors.
- 3 Vulnerable – At moderate risk of extinction or elimination due to a fairly restricted range, relatively few populations or occurrences, recent and widespread declines, threats, or other factors.
- 4 Apparently Secure – At fairly low risk of extinction or elimination due to an extensive range and/or many populations or occurrences, but with possible cause for some concern as a result of local recent declines, threats, or other factors.
- 5 Secure – At very low risk or extinction or elimination due to a very extensive range, abundant populations or occurrences, and little to no concern from declines or threats.
- NR Not yet ranked- This species has not yet been evaluated.

Local conservation ranks assigned by a municipality, region or conservation authority may differ from the above.

Allelopathy – The release of secondary compounds of plants that effect the growth and development of nearby plants

Corm – roundish underground stem.

Cuneate – Wedge-shaped, with straight but not parallel margins.

Dorsal Sepal – Upper sepal of an orchid flower, normally directly above the lip/labellum.

Edaphic – Relating to the soil.

Ellipsoid – A three-dimensional shape that is elliptical in sections through the long axis.

Elliptic – Longer than wide, broadest at the middle, tapering ±equally toward both ends.

Extant – A population, subpopulation or occurrence that is still present

Extirpated – A species, population, subpopulation or occurrence that was present in an area, but is now no longer present

Facultative upland species- A species of plant that occasionally occurs in wetlands, but usually occurs in non-wetlands (estimated 1% - 33% probability).

Filiform – Thread-like; very slender and roughly as broad as thick.

Genus – A group of one or more similar species. Taxonomic rank between family and species.

Germination – The beginning process of the seed developing and growing into a plant

Herbaceous (Herb) – Vascular plants that do not does not develop woody tissue, e.g. a dandelion.

Hummock – Small mounds rising above the general level of a marsh or bog.

Inflorescence – An entire flower cluster, including pedicels and bracts.

Irruptive years – Years where population abundance is drastically higher than previously recorded.

Irregular flowers – Can only be divided into similar halves through one plane, e.g. orchid flowers.

Keeled – A ridge centrally located on the long axis of a structure, such as a leaf, sepal or an achene.

Lanceolate – Longer than broad, broadest in the lower half, tapering to the tip like the shape of a lance.

Lateral – Attached to the side of an organ.

Linear – Long and narrow, sides mostly parallel, e.g. blade of grass.

Lip (Labellum) – A modified petal that is different from the other two in the Orchidaceae family, typically the lowest petal.

Mesic – Moist, moderate moisture or water supply.

Mucronate – Short, sharp, slender point.

Mycorrhizal – Relating to fungi that grow in association with the roots of plants forming a relationship that increases the fitness of both the plants and fungi.

Oblanceolate – Longer than broad, broadest closer to the tip than the base. The reverse of lanceolate.

Oblong – Longer than wide with ± parallel sides, not as elongate as linear.

Obovate – Teardrop shaped; stem attaches to tapering end. The reverse of ovate.

Obtuse – With the sides or margins converging on more than a 90˚ angle.

Orbiculate – Circular in outline.

Ovary – The lower portion of the pistil, usually expanded, in which the seed(s) are produced; ripens into the fruit.

Ovate – Egg-shaped, tapering point towards leaf tip.

Pedicel – The stalk of an individual flower, spikelet or head.

Peduncle – The stalk of an entire inflorescence, or of a single flower when there only is one.

Pendent – Hanging or drooping.

Perennial – Living for three or more years.

Pollinia – Mass of pollen grains produced by one anther but stay together when transferred, common in Orchidaceae and milkweed species.

Protocorms – Tuber-shaped body with rhizoids (root-like growths) that is produced by the young seedlings of various orchids and other species that are associated with mycorrhizal fungi.

Resupinate – Leaves or flowers that are twisted 180˚ so that they are inverted.

Revolute – With margins rolled back or under.

Rhizome – An underground stem that runs horizontally. It can send up roots and shoots from its nodes.

Sepals – Part of the calyx, the outermost whorl of the flower.

Serrulate – Finely serrate; sharp forward pointing teeth along the margins.

Stamen – Male or pollen producing structures of a flower, composed of a filament and an anther.

Subacute – Moderately acute or approaching acute.

Subtruncate – Nearly truncate.

Surficial – On the earth's surface

Symbiont – An organism benefiting from a symbiotic relationship with another organism

Terrestrial – On or growing from the ground, not aquatic.

Tribe – The taxonomic ranking above genus but below family.

Truncate – With a squared-off end.

Tubercles – Small enlargement at the tip of an appendage; wart-like.

Tubular – Long, round, hollow; like a tube.

Winged – A flat, ± thin extension on the edge of a surface of an organ.

Winter dormancy – Reduced metabolic activity during the winter season. Above-ground tissues of herbaceous plants may die-back to the soil.

6. List of Abbreviations

COSEWIC: Committee on the Status of Endangered Wildlife in Canada
COSSARO: Committee on the Status of Species at Risk in Ontario
ECCC: Environment and Climate Change Canada
ESA: Ontario's *Endangered Species* Act, 2007
MECP: Ministry of the Environment, Conservation and Parks
MNRF: Ministry of Natural Resources and Forestry
NCC: Nature Conservancy of Canada
SARA: Canada's *Species at Risk Act*
SARO List: Species at Risk in Ontario List

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APPENDIX 1 | Presence/ No Detection Data Sheet

Purple Twayblade: Presence/ No Detection Survey Data Form

DATE _____

SURVEYOR (S) _____

_____

START TIME_____ **END TIME** _____

TOTAL TIME _____ **PERSON HOURS** _____

LOCATION DESCRIPTION

SITE NAME _____

COUNTY/DISTRICT _____

NEAREST TOWN/CITY _____

CENTROID_____

_____

_____

_____

SURVEY TYPE ☐ Transect (Transect Width _____m) ☐ Intuitive Search

WAS PURPLE TWAYBLADE LOCATED? ☐ Yes ☐ No **ABUNDANCE** _____

SEARCH EFFORT COMMENTS:

_____

_____

_____

HABITAT NOTES:

_____

_____

_____

_____

OTHER SAR/ SPECIES OF CONSERVATION CONCERN:

SPECIES	COORDINATES	ABUNDANCE

NON-NATIVE SPECIES:

SPECIES	COORDINATES	ABUNDANCE

SITE NOTES (MANAGEMENT, THREATS, ETC.):

_____

_____

_____

_____

_____

Purple Twayblade: Presence/ No Detection Survey Data Form

Date ______________________________ Surveyor (s) ____

GPS Accuracy__________

Site Name ____ Site Location ____

Individual/Patch Locations

Plant/ Patch ID #	Easting	Northing	Patch Size	Total # Plants	# Flowering Plants	# Non-Flowering Plants	Notes (indication of poor health, insects, etc.)

Notes

APPENDIX 2 | Long-term Monitoring Data Sheet

Purple Twayblade: Long-Term Monitoring Site Data Form

Date _____

Surveyor (s) _____

_____

Start Time_____ **End Time** _____

Total Time _____ **Person Hours** _____

Site Name _____

County/District _____

Nearest Town/City _____

Centroid_____

Location Description _____

_____

_____

Was Purple Twayblade Re-Located? ☐ Yes ☐ No **Abundance** _____

Are Any uncertain Individuals Present on site? ☐ Yes ☐ No **Abundance** _____

PURPLE TWAYBLADE HABITAT DATA

Vegetation Community

HT Codes: 1 = >25m 2 = 10-25m 3 = 2-10m 4 = 1-2m 5 = 0.5-1m 6 = 0.2-0.5m 7 = <0.2m

CVR Codes: 0 = NONE 1 = 1-10% 2 = 10-25% 3 = 25-60% 4 = >60%

Layer	HT	CVR	Species in Order of Decreasing Dominance (>> Much Greater Than; > Greater Than; = About Equal To)
Canopy			
Sub-Canopy			
Understory			
Ground			

Percent Cover Estimates Thatch: _____ Leaf Litter: _____ Bare Earth: _____ Rock: _____

Woody Debris _____ Non-Native Species: _____

Non-native Species: _____

_____

Habitat Notes: _____

_____

_____

THREATS

Threat Type	Scope	Severity	Threat Impact	Timing	Comments

Site Notes (Management, Threats, ETC.):

_____

_____

_____

_____

_____

Marker Description:

Purple Twayblade: Long-term Monitoring Data Form

DATE _____

SURVEYOR (S) _____

SITE _____

OCCURRENCE SIZE _____

NUMBER OF PURPLE TWAYBLADE _____

NUMBER OF UNCERTAIN PLANTS _____

NUMBER OF RELOCATED PLANTS _____

NUMBER OF NEW PLANTS _____

INDIVIDUAL LOCATIONS

PLANT/ PATCH ID #	R OR N?[1]	PATCH SIZE	TOTAL # PLANTS	# FLOWERING PLANTS	# NON-FLOWERING PLANTS	NOTES (INDICATION OF POOR HEALTH, INSECTS, TAG CONDITION, GPS IF NEW, ETC.)

NOTES

[1] R= Relocated individual. N= New individual.

Purple Twayblade: Long-term Monitoring Patch Data Form

PATCH LOCATION _____ _____ _____

MARKED WITH _____

PATCH MEASUREMENTS FROM CENTROID (M)

NW _____ **N** _____ **NE** _____ **E** _____

SE _____ **S** _____ **SW** _____ **W** _____

PATCH AREA _____

DRAWING OR IMAGE AND MEASUREMENTS OF PATCH

NOTES

APPENDIX 3 | Voucher Specimen Data Sheet

Collector's Name ______________________________
Collection Date ______________________________
Collection Number ____________________________
Photo Number(s) ______________________________
Species ______________________________________
Location _____________________________________
Location Description
____
____
____
____
GPS co-ordinates _____ _______________ _______________
Site/Community Description
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Notes
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Collector's Name ______________________________
Collection Date ______________________________
Collection Number ____________________________
Photo Number(s) ______________________________
Species ______________________________________
Location _____________________________________
Location Description
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GPS co-ordinates _____ _______________ _______________
Site/Community Description
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Notes
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Collector's Name ______________________________
Collection Date ______________________________
Collection Number ____________________________
Photo Number(s) ______________________________
Species ______________________________________
Location _____________________________________
Location Description
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GPS co-ordinates _____ _______________ _______________
Site/Community Description
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Collector's Name ______________________________
Collection Date ______________________________
Collection Number ____________________________
Photo Number(s) ______________________________
Species ______________________________________
Location _____________________________________
Location Description
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Site/Community Description
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