

# Recovery Strategy for the Purple Twayblade (*Liparis liliifolia*) in Canada

## Purple Twayblade



2016



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<sup>1</sup> <http://www.registrelep-sararegistry.gc.ca>

## Preface

The federal, provincial, and territorial government signatories under the [Accord for the Protection of Species at Risk \(1996\)](#)<sup>2</sup> agreed to establish complementary legislation and programs that provide for effective protection of species at risk throughout Canada. Under the *Species at Risk Act* (S.C. 2002, c.29) (SARA), the federal competent ministers are responsible for the preparation of recovery strategies for listed Extirpated, Endangered, and Threatened species and are required to report on progress within five years after the publication of the final document on the SAR Public Registry.

The Minister of the Environment is the competent minister under SARA for the Purple Twayblade and has prepared this strategy, as per section 37 of SARA. To the extent possible, it has been prepared in cooperation with the Province of Ontario and the Province of Quebec.

Success in the recovery of this species depends on the commitment and cooperation of many different constituencies that will be involved in implementing the directions set out in this strategy and will not be achieved by Environment Canada, or any other jurisdiction alone. All Canadians are invited to join in supporting and implementing this strategy for the benefit of the Purple Twayblade and Canadian society as a whole.

This recovery strategy will be followed by one or more action plans that will provide information on recovery measures to be taken by Environment Canada and other jurisdictions and/or organizations involved in the conservation of the species. Implementation of this strategy is subject to appropriations, priorities, and budgetary constraints of the participating jurisdictions and organizations.

The recovery strategy sets the strategic direction to arrest or reverse the decline of the species, including identification of critical habitat to the extent possible. It provides all Canadians with information to help take action on species conservation. When the recovery strategy identifies critical habitat, there may be future regulatory implications, depending on where the critical habitat is identified. SARA requires that critical habitat identified within a national park named and described in Schedule 1 to the *Canada National Parks Act*, the Rouge National Urban Park established by the *Rouge National Urban Park Act*, a marine protected area under the *Oceans Act*, a migratory bird sanctuary under the *Migratory Birds Convention Act, 1994* or a national wildlife area under the *Canada Wildlife Act* be described in the *Canada Gazette*, after which prohibitions against its destruction will apply. For critical habitat located on other federal lands, the competent minister must either make a statement on existing legal protection or make an order so that the prohibition against destruction of critical habitat applies. For any part of critical habitat located on non-federal lands, if the competent minister forms the opinion that any portion of critical habitat is not protected by provisions in or measures under SARA or other Acts of Parliament, or the laws of the province or territory, SARA requires that the Minister recommend that the Governor in Council make an order to prohibit destruction of critical habitat. The discretion to protect critical habitat on non-federal lands that is not otherwise protected rests with the Governor in Council.

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<sup>2</sup> <http://registrelep-sararegistry.gc.ca/default.asp?lang=en&n=6B319869-1#2>

## Acknowledgments

The original draft of this recovery strategy was developed by John Ambrose (Cercis Consulting), Gerry Waldron (private consultant) and the Ontario Tallgrass Communities Recovery Team, with assistance from the late Dr. Jane Bowles (formerly of the University of Western Ontario), Graham Buck (now Ontario Ministry of Natural Resources and Forestry (OMNRF)), Peter Carson (Pterophylla Native Plants), Lindsay Rodger (Parks Canada Agency), Ken Tuininga (Environment Canada, Canadian Wildlife Service – Ontario region (CWS-ON)), Allen Woodliffe (formerly Ontario Ministry of Natural Resources (OMNR)) and Holly Bickerton (private consultant).

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## Executive Summary

Purple Twayblade (*Liparis liliifolia*) is listed as Endangered on Schedule 1 of the federal *Species at Risk Act* (SARA). It is a terrestrial orchid that grows about 25 cm tall and has two fleshy oval leaves. In late May through early July, a flowering stalk arises between the leaves bearing several translucent, purplish-brown flowers.

In Canada, Purple Twayblade occurs across southern Ontario, and recent discoveries in eastern Ontario and western Quebec have extended the known Canadian range eastward. There have been 18 Purple Twayblade populations documented in Canada, of which up to 11 are considered extant. The total estimated population in Canada is 1,200 plants, though most populations consist of fewer than 30 plants. Purple Twayblade is an inconspicuous species that may be under-reported in Canada. It is estimated that 5 to 10% of the species' global range is in Canada.

Purple Twayblade is most often found in open and semi-open areas, although it has been reported in a broad variety of habitats and soil conditions (e.g., sand, silt, clay loam, and in soils that range from strongly acidic to neutral) across its range. In Canada, it has been reported in open oak woodland and savanna, tallgrass prairie, deciduous forest, shrub-thicket, shrub alvar, forested swamps (deciduous and mixed deciduous-coniferous) and conifer plantations. Purple Twayblade is a colonizing orchid that can quickly establish large colonies following a disturbance and then decrease to just a few plants within a matter of years as habitat conditions become less suitable. Purple Twayblade is a symbiotic species that associates with mycorrhizae (microscopic fungi) in the soil and the presence of this associate is essential for successful germination and plant development.

The primary threats to Purple Twayblade are habitat loss due to development (e.g., urban and agricultural), invasive plants and alteration of the natural disturbance regime (e.g., fire suppression). Additional threats identified to the Canadian population of Purple Twayblade include: herbivory and habitat alteration by invasive invertebrates, prolonged flooding, herbivory by White-tailed Deer (*Odocoileus virginianus*), and herbicide, fungicide and pesticide application. The broad strategies to be taken to address the threats to the survival and recovery of the species are presented in the section on Strategic Direction for Recovery (section 6.2).

Although there are unknowns regarding the feasibility of recovery, in keeping with the precautionary principle, a full recovery strategy has been prepared as would be done when recovery is determined to be feasible. The population and distribution objectives for the Purple Twayblade in Canada are to: maintain the abundance and number of extant populations and corresponding sub-populations; where biologically and technically feasible, increase population abundance and restore historical populations, and; maintain the approximate distribution of the extant populations and corresponding sub-populations.

Critical habitat for Purple Twayblade is partially identified in this recovery strategy, based on habitat suitability and habitat occupancy. A schedule of studies (section 7.2) has been developed and outlines the activities required for identification of additional critical habitat necessary to support the population and distribution objectives.

One or more action plans will be prepared for Purple Twayblade by December 2023.

## Recovery Feasibility Summary

Based on the following four criteria that Environment Canada uses to establish recovery feasibility, there are unknowns regarding the feasibility of recovery of the Purple Twayblade. In keeping with the precautionary principle, this recovery strategy has been prepared as per section 41(1) of SARA, as would be done when recovery is determined to be feasible. This recovery strategy addresses the unknowns surrounding the feasibility of recovery.

1. Individuals of the wildlife species that are capable of reproduction are available now or in the foreseeable future to sustain the population or improve its abundance.

**Yes.** There are currently up to 11 extant (i.e., existing) populations (element occurrences<sup>3</sup>) of Purple Twayblade in Canada, and the total number of plants is estimated to be 1,200 in any given year (COSEWIC 2010). Purple Twayblade is known as a colonizing orchid that may rapidly populate new areas (Sheviak 1974; Mattrick 2004). Plants at several Canadian populations have recently been observed in flower and fruit, indicating that these populations are able to reproduce (COSEWIC 2010). Purple Twayblade is present in bordering American states and is common in many areas of the eastern United States (Appendix B). Propagules<sup>4</sup> from Purple Twayblade in these areas could be considered for reintroduction, if biologically and technically feasible and required.

2. Sufficient suitable habitat is available to support the species or could be made available through habitat management or restoration.

**Unknown.** Purple Twayblade inhabits a wide variety of habitats in Canada, including oak savanna, shrub thicket, tallgrass prairie, early-successional deciduous forests, conifer plantations, and forested swamps (deciduous and mixed deciduous-coniferous) (COSEWIC 2010). The recent discovery of populations in eastern Ontario and western Quebec suggests that suitable habitat may exist in areas previously thought to be outside the geographical range of this species. According to McCormick et al. (2012), the presence and abundance of a specific soil fungus appears to be a determining factor for growth and germination of Purple Twayblade, and is more important than (for example) the successional forest stage. Thus, it is possible that the primary determinant of suitable habitat is the distribution of the appropriate soil fungus of the genus *Tulasnella*. Very little is known about this fungus' distribution and ecology in Canada.

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<sup>3</sup> Element occurrence: an area of land and/or water in which a species or natural community is, or was present. Throughout this document, the term "population" is considered to be synonymous with the term "element occurrence" as used by the provincial Conservation Data Centres (CDC) and NatureServe (i.e., populations that are more than 1 km apart) following standard guidelines developed by NatureServe for vascular plants.

<sup>4</sup> Any of various structures that can give rise to a new individual organism, especially parts of a plant that serve as means of vegetative reproduction, such as corms, tubers, offsets, or runners. Seeds and spores are also propagules.

Techniques to restore habitat by inoculating soil with the appropriate fungi are currently experimental but may be developed (see #4 below).

3. The primary threats to the species or its habitat (including threats outside Canada) can be avoided or mitigated.

**Yes.** The primary threats to Purple Twayblade are habitat loss due to development (e.g., agricultural, residential and urbanization), invasive plants, and alteration of the natural disturbance regime (e.g., fire suppression). Threats posed by development can be avoided through the use of municipal planning policies and in some cases, land acquisition or other non-regulatory protection techniques. Land protection for conservation purposes is underway in southern Canada, with national and local land trusts prioritizing the habitat of species at risk for acquisition and other conservation options. As such, several properties where Purple Twayblade occurs have recently been acquired by conservation groups or public agencies (COSEWIC 2010). In many cases, threats posed by invasive species can be mitigated with programs to avoid or control this primary threat. Lack of disturbance results in forest succession and eventual shading of Purple Twayblade plants. This process may be halted or reversed by using techniques such as prescribed burning and canopy thinning, which may benefit Purple Twayblade by maintaining the openness of the canopy and suitable habitat conditions.

4. Recovery techniques exist to achieve the population and distribution objectives or can be expected to be developed within a reasonable timeframe.

**Yes.** There are several techniques that exist or are in development, and could aid in the recovery of Purple Twayblade. Techniques to inoculate soil with mycorrhizal associates (soil fungus) to increase Purple Twayblade protocorm<sup>5</sup> and seedling growth are in development (McCormick et al. 2012). Prescribed burning may help to restore and maintain early-successional habitat conditions, although the effects of fire on the survival and regenerative capacity of Purple Twayblade, or its associated fungi, are not well known (Mattrick 2004). Successful techniques have also been developed to propagate Purple Twayblade seeds *in vitro* and transplant seedlings (Rasmussen and Whigham 1998; S. Weber, pers. comm. 2014), as well as to germinate seeds *in situ* (McCormick et al. 2012), although cultivation is not recommended within the current scope of population and distribution objectives.

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<sup>5</sup> A protocorm is a tuber-shaped body with rhizoids (small root structures) that is produced by young seedlings of certain types of orchids and other plants that have mycorrhizal associates.



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## 1. COSEWIC\* Species Assessment Information

**Date of Assessment:** November 2010

**Common Name (population):** Purple Twayblade

**Scientific Name:** *Liparis liliifolia*

**COSEWIC Status:** Threatened

**Reason for Designation:** This small inconspicuous orchid extends across southern Ontario to southwestern Quebec as a series of scattered populations. The discovery of several new populations in recent years has extended its known range in Canada. The few individuals present in the majority of the populations and the overall small size of the entire Canadian population places the species at continued risk from chance events.

**Canadian Occurrence:** Ontario, Quebec

**COSEWIC Status History:** Designated Threatened in April 1989. Status re-examined and designated Endangered in April 1999 and in May 2001. Status re-examined and designated Threatened in November 2010.

\* COSEWIC (Committee on the Status of Endangered Wildlife in Canada)

## 2. Species Status Information

The global conservation rank for Purple Twayblade is secure<sup>6</sup> (G5). In the United States, Purple Twayblade is a common species in the eastern and mid-western states and the national conservation rank is secure (N5) (NatureServe 2014, Appendix B). In Canada, Purple Twayblade is found in the provinces of Ontario and Quebec. The national conservation rank is imperiled<sup>7</sup> (N2) and the subnational conservation rank is imperiled (S2) for Ontario and critically imperiled<sup>8</sup> (S1) for Quebec (NatureServe 2014).

Purple Twayblade is currently listed as Endangered<sup>9</sup> on Schedule 1 of the federal *Species at Risk Act* (SARA). In Ontario, Purple Twayblade is listed as Endangered<sup>10</sup>

<sup>6</sup> Common, widespread and abundant.

<sup>7</sup> At high risk of extinction or elimination due to very restricted range, very few populations, steep declines, or other factors.

<sup>8</sup> Extreme rarity (often 5 or fewer occurrences) or because of some factor(s) such as very steep declines making it especially vulnerable to extirpation from the province.

<sup>9</sup> A wildlife species facing imminent extirpation or extinction. Note: the species status may be reclassified under SARA into a lower risk category (i.e., Threatened) based on COSEWIC's last examination and change (COSEWIC 2010).

under the provincial *Endangered Species Act, 2007* (ESA). In Quebec, the species is likely to be designated as threatened or vulnerable under the provincial *Act respecting threatened or vulnerable species* (Centre de données sur le patrimoine naturel du Québec 2015 (CDPNQ 2015)). It is estimated that 5 to 10% of the species' global range is in Canada (Figure 1).

### 3. Species Information

#### 3.1 Species Description

Purple Twayblade is an inconspicuous terrestrial orchid that typically grows between 10 cm and 25 cm high. A single flowering stalk arises in late May through early July from between two fleshy, oval-shaped leaves. Between 5 and 30 flowers are arranged along the stalk. Each flower consists of linear, tubular petals that are purple to brown in colour (10 - 12 mm long), with a similarly coloured translucent lip (10 - 13 mm long and 8 - 10 mm wide). Three greenish-white narrowly lanceolate sepals<sup>11</sup> surround these petals (COSEWIC 2010). The lip is streaked with a fine network of reddish-purple veins. The fruit of Purple Twayblade is a capsule, usually about 1.5 cm long, which contains a large number of dust-like seeds (Gleason and Cronquist 1991; Holmgren 1998). Seeds may be widely dispersed on wind currents (Dressler 1981).

#### 3.2 Population and Distribution

Purple Twayblade is endemic to North America, and is common throughout the eastern and mid-western states. It extends from northern New England and New York west to Minnesota and south to the upland regions of Georgia and Alabama (Figure 1). In Canada, the majority of populations are within southwestern Ontario, with a concentration in Essex County and the Windsor area. However, two newly discovered populations in eastern Ontario and western Quebec extend the species' known Canadian range eastward (Figure 2).

In Canada, 18<sup>12</sup> populations<sup>13</sup> of Purple Twayblade have been documented (Figure 2; Appendix C). Seventeen of these populations occur, or occurred, in Ontario; one population occurs in Quebec. Three are considered extirpated (i.e., no longer exist) and four are considered historical (i.e., not confirmed in over 20 years). Up to 11 extant populations are believed to exist. The uncertainty about the number of extant

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<sup>10</sup> A species that lives in the wild in Ontario but is facing imminent extinction or extirpation.

<sup>11</sup> The leaf like structures composing the outermost part of the flower that are narrow and taper to a point.

<sup>12</sup> The number of populations reported in COSEWIC (2010) is 23 with 10-12 considered extant. The difference in the number presented here represents a re-grouping of the populations based on NatureServe's (2002) methods for describing populations (and sub-populations) of vascular plants rather than a decrease in the number of Purple Twayblade occurrences.

<sup>13</sup> Populations are considered to be independent if separated by one kilometre or more of inappropriate habitat, and groupings of plants separated by less than one kilometre are considered sub-populations (NatureServe 2002).

populations is due to the fact that when last visited in 2008, plants were not detected at the locations of three populations (though they are still classified as extant by Ontario's Conservation Data Centre) (Appendix C). At two of these locations, invasive species have become established or the habitat had become overgrown and/or shaded. Although it is unknown whether or not Purple Twayblade, like many other orchid species, can persist either vegetatively (i.e., in a dormant state underground) or in the seed bank for long periods of time; it is seed bank forming and there is evidence that seeds can remain dormant for over four years then germinate when exposed to a mycorrhizal associate (Whingham et al. 2006). If this is the case, habitat restoration activities could be investigated to possibly restore the two populations. As for the third population (Frontenac Provincial Park), a beaver dam in 2004 flooded the area where an estimated 400-500 Purple Twayblade plants were growing the previous year. No Purple Twayblade plants have been observed there since that time, despite removal of the beaver dam in 2007 (which has since been rebuilt). It is unknown whether Purple Twayblade (or its fungal associate) can survive prolonged submersion, this population, in particular, would benefit from continued monitoring to determine its presence / absence, the availability of suitable habitat (including the fungal associate) and/or opportunities for restoration (species and/or habitat).

Two new occurrences of Purple Twayblade were discovered in the Windsor area, one in 2012 and the other in 2013 (AMEC 2014). The naturally-occurring plants were found within restoration sites established for the Herb Gray Parkway (HGP) project<sup>14</sup>. These occurrences have not yet been assessed by the Natural Heritage Information Centre (NHIC). However, based on distance to other extant populations, it is likely that they would be grouped (i.e., sub-population) within the larger Ojibway Prairie Complex and Area population (Appendix C). They have therefore been grouped as such here.

The total Canadian population is estimated to be 1,200 plants in any one year (Appendix C). Though most Ontario populations are small (fewer than 30 plants), the Morgan Arboretum population in Quebec is comparatively quite large (more than 900 plants) (Appendix C). In Ontario, some of the small population size may be attributed to survey effort; some populations were only partially counted during the survey period and it is likely that the estimate is also conservative because not all populations (and sub-populations) were visited (COSEWIC 2010). In addition, outside the flowering and fruiting period, Purple Twayblade is inconspicuous and may be mistaken for the related, and more common, Loesel's Twayblade (*Liparis loeselii*). As such, under similar circumstances, it is also equally possible Loesel's Twayblade may also be mistaken for Purple Twayblade leading to false positives and inaccurate population estimates.

Trends in population size are very difficult to determine for this species, and detailed information is not available. To date, none of the Purple Twayblade populations have been monitored sufficiently to detect population trends. It has been suggested that, like

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<sup>14</sup> The Rt. Hon. Herb Gray Parkway is a major highway infrastructure project that will form part of the transportation corridor connecting Highway 401 in Ontario to Interstate 75 in Michigan.

many other orchids, Purple Twayblade plants may remain dormant in the soil when conditions are unfavourable (White 2001), although there is no evidence for this from research on this species (Mattrick 2004). Purple Twayblade is known as a colonizing orchid that may rapidly populate new areas and then decrease rapidly to only a few plants, as habitat becomes unsuitable (Sheviak 1974; Mattrick 2004).



**Figure 1. Global distribution of Purple Twayblade (adapted from COSEWIC 2010).**



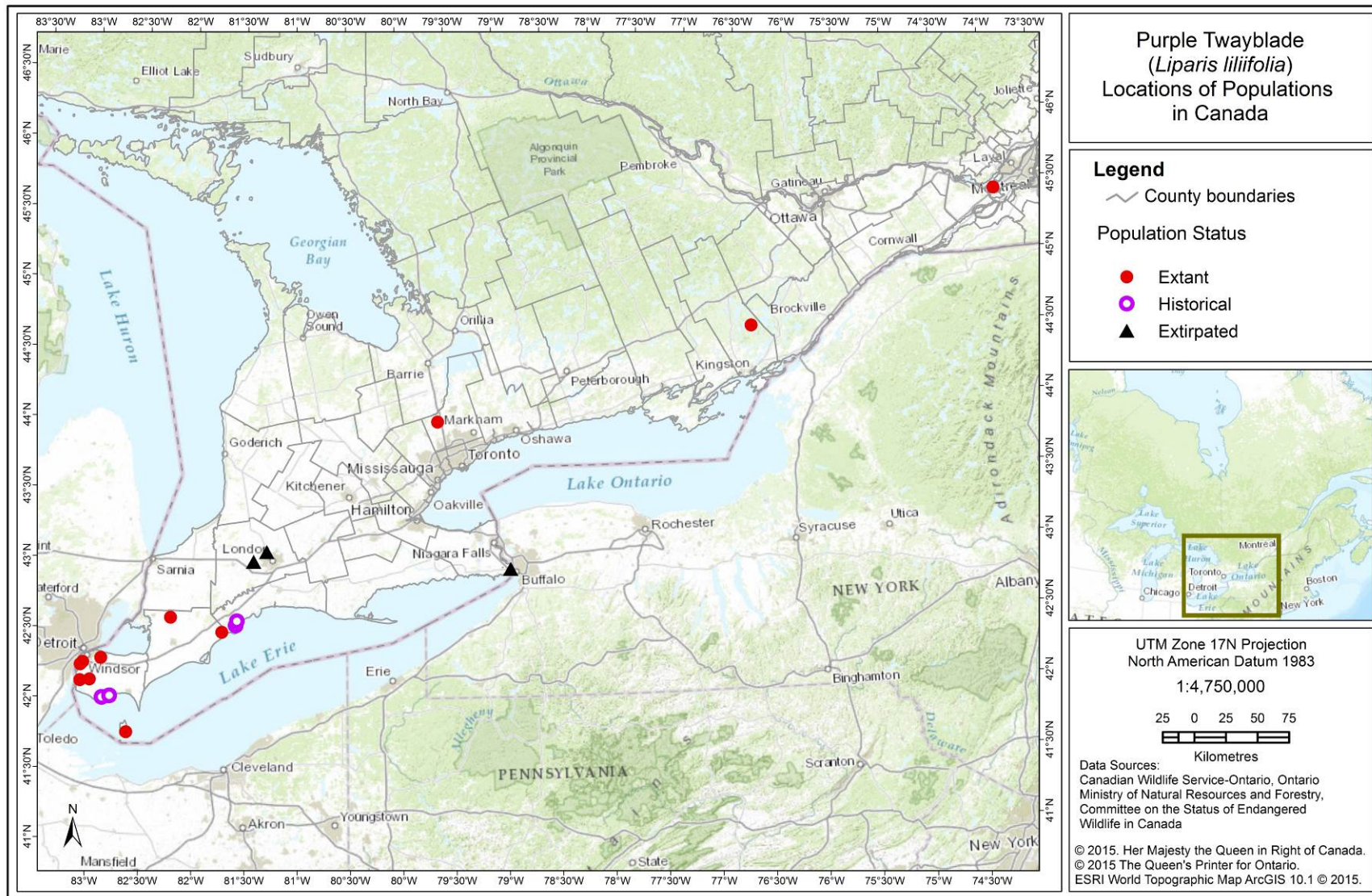


Figure 2. Distribution of the Purple Twayblade in Canada.

### 3.3 Needs of the Purple Twayblade

#### ***Vegetation associates and abiotic conditions***

In the central part of its North American range, Purple Twayblade has been found in old fields, rich hardwood forests, floodplains and sand ridges in prairie habitats (Sheviak 1974; Case 1987). In Canada, it was formerly thought to be a species found mainly in open oak woodland and savanna (Allen 1989), but in recent decades has been reported from a wider range of habitats, from mixed deciduous forest, shrub-thicket, shrub alvar, forested swamps (deciduous and mixed deciduous-coniferous), tallgrass prairie and coniferous plantations (Allen 1989; White 2001; Buck and Dobbyn 2007; Ambrose et al. 2004; White 2008; A. Godbout, pers. comm. 2010; COSEWIC 2010; AMEC 2014). In the core of its range in the United States, it is considered a somewhat weedy species, capable of colonizing early- to mid-successional habitats (Sheviak 1974; Dressler 1981; Homoya 2012).

Purple Twayblade is found in habitats with canopies of various densities. However, it is reported to prefer open to semi-open canopies, and declines in abundance and reproductive success have been observed as conditions succeed to closed canopy (Sheviak 1974). In Canada, the species has been reported in large numbers from at least three populations (or sub-populations) with closed forested canopies (i.e., mature deciduous forest or swamp) (COSEWIC 2010). Mattrick (2004) suggests that gaps created by wind throws, may result in increased sunlight penetration and species' establishment in forested habitats.

Although this orchid appears to favour mesic<sup>15</sup> to moist conditions, it is able to tolerate wet and dry habitats (Mattrick 2004). In the United States and Canada, Purple Twayblade is not generally found in wetlands (NHIC 1995; Mattrick 2004). Most Canadian populations are found on well-drained upper slopes. However, two populations have been observed in deciduous or mixed wood swamps (White 2008; A. Godbout, pers. comm. 2010). One of these populations represents the largest population known in Canada (i.e., the Morgan Arboretum in Quebec; Appendix C). Purple Twayblade is also able to tolerate a wide variety of soil conditions, such as sand, silt, and clay loam, as well as soils that have a pH range from strongly acidic to neutral (Sheviak 1974; Smith 1993; Homoya 2012).

#### ***Mycorrhizae***

An important component of the habitat of Purple Twayblade is the presence and abundance of a mycorrhizal soil fungus<sup>16</sup> that supplies nutrients to the orchid. This

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<sup>15</sup> Describing sites that are neither humid nor very dry; represents the average moisture conditions for a given climate.

<sup>16</sup> A mycorrhizal associate is a symbiotic fungus that shares a close physical association with the roots of a host plant. Both organisms appear to benefit; a host plant with roots infected by the fungus appears to



association is critical to the orchid's existence. Orchid seeds and seedlings require this fungal associate to provide nutrients until the plant is capable of photosynthesis (Whigham et al. 2006). The specificity of this symbiotic relationship differs among orchids; some taxa are highly specialized, while others are capable of a greater range of variation in the fungal association. In the case of Purple Twayblade, there is strong evidence that the species in all stages of development requires a very specific fungal associate that shows little genetic variation over the range of the species (Whigham, pers. comm. cited in Mattrick 2004; McCormick et al. 2004). McCormick et al. (2004, 2006) found that fungi isolated from Purple Twayblade in the United States belonged to a single clade<sup>17</sup> of soil fungi in the genus *Tulasnella*, with little variation. Whigham et al. (2006) identified the fungus from an embryonic Purple Twayblade protocorm as the same fungus that associates with adult plants, suggesting a high degree of specificity for this species through all stages of development.

In experimental trials *in situ*, McCormick et al. (2012) found the successful germination of Purple Twayblade was almost entirely limited by the distribution and abundance of the fungal associate (*Tulasnella* spp.), and its presence was more important to germination than forest successional stage (McCormick et al. 2012).

In general, little information exists about the distribution of mycorrhizal fungi in southern Ontario and Quebec, or about the distribution of the specific fungal associate upon which Purple Twayblade apparently depends. However, it is possible to determine whether an area is occupied by the fungal associate(s), but requires molecular analysis of deoxyribonucleic acid (DNA) extracted from soil samples (McCormick et al. 2012).

### **Pollinators**

Purple Twayblade is incapable of self-fertilization, meaning that it requires cross-pollination<sup>18</sup> to produce viable seed (Whigham et al. 2002). Fruit set in this species is reportedly very low (Whigham and O'Neill 1991 cited in Argue 2012). Although the mechanism of pollination and the pollinators of Purple Twayblade are unknown, the genus *Liparis* is generally pollinated by flies (Diptera), a non-specialized type of insect (Argue 2012). In comparison to other insects, Dipterans are thought to be poor pollinators, meaning that although they may visit plants frequently, the visits do not always result in pollination. The group of Diptera that frequently visit Purple Twayblade flowers is within the family Sarcophagidae (flesh flies), but it is not known if these flies successfully pollinate flowers, and if so, whether they are the primary species responsible for pollination (Christensen 1994, cited in Mattrick 2004). Given the growing appreciation for the role flies play as pollinators (Orford et al. 2015); future research into the pollinators of Purple Twayblade in Canada may be beneficial.

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take up soil nutrients more efficiently than an uninfected root (Allaby 1992). This is a common requirement in members of the Orchid (Orchidaceae) family.

<sup>17</sup> A taxonomic group of organisms classified together.

<sup>18</sup> Cross-pollination refers to the transfer of pollen from the male parts (styles) of one plant to the female part (stigma) of a different plant (Allaby 1992).

### 3.4 Biologically Limiting Factors

Purple Twayblade is biologically limited within its Canadian range for various reasons. It requires cross-pollination to produce viable seed, and hand-pollination experiments have demonstrated that plants in close proximity to one another are less likely to produce viable seed (Whigham and O'Neill 1991 as cited in Mattrick 2004). As a result, there may be a reduced opportunity for cross-pollination among Canadian populations, many of which are small and widely separated. In Maryland, small, isolated populations have shown evidence of severe inbreeding depression <sup>19</sup>(Mattrick 2004). It is possible that inbreeding depression also affects this species in Canada.

Purple Twayblade's association with a specific mycorrhizal fungus likely also limits its distribution and abundance in Canada. Very little is known about the fungus' distribution in Canada.

Currently, the Morgan Arboretum population in Quebec has the largest known abundance of Purple Twayblade in Canada. As such, additional research would be beneficial to better understand what it is about this location that favours the species.

In addition, most of the extant populations occupy an area of less than a few square metres. Such populations may be vulnerable to local, chance events such as storms and flooding, as has occurred at the Frontenac Provincial Park population.

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<sup>19</sup> The loss of vigor and general health that sometimes characterizes organisms that are the product of inbreeding.

## 4. Threats

### 4.1 Threat Assessment

**Table 1. Threat Assessment Table.**

Threat*	Level of Concern <sup>1</sup>	Extent	Occurrence	Frequency	Severity <sup>2</sup>	Causal Certainty <sup>3</sup>
<b>Habitat Loss or Degradation</b>						
Residential development and urbanization	High	Localized	Historic, Current, Anticipated	Recurrent	High	High
Agricultural development	Medium	Localized	Historic, Anticipated	Recurrent	High	High
<b>Changes in Ecological Dynamics or Natural Processes</b>						
Alteration of the natural disturbance regime	High	Widespread	Historic, Current, Anticipated	Continuous	Medium	High
<b>Invasive Species</b>						
Invasive plants	Medium	Widespread	Current	Continuous	Moderate	Medium
Invasive invertebrates	Low**	Widespread	Current	Continuous	Unknown	Low
<b>Natural Processes or Activities</b>						
Flooding caused by Beaver ( <i>Castor canadensis</i> ) activity	Low**	Localized	Current	Recurrent	High	High
Herbivory	Low**	Widespread	Anticipated	Unknown	Low	Low
<b>Pollution</b>						
Herbicide, fungicide and pesticide application	Unknown	Localized	Historic, Anticipated	Recurrent	Unknown	Low

<sup>1</sup> Level of Concern: signifies that managing the threat is of (high, medium or low) concern for the recovery of the species, consistent with the population and distribution objectives. This criterion considers the assessment of all the information in the table.

<sup>2</sup> Severity: reflects the population-level effect (High: very large population-level effect, Moderate, Low, Unknown).

<sup>3</sup> Causal certainty: reflects the degree of evidence that is known for the threat (High: available evidence strongly links the threat to stresses on population viability; Medium: there is a correlation between the threat and population viability e.g. expert opinion; Low: the threat is assumed or plausible).

\* Threats are listed in decreasing level of significance.

\*\* Threats with a low Level of Concern are listed and described but may not be specifically addressed in the recovery approaches.

## 4.2 Description of Threats

Because of the emerging information that suggests that the presence and abundance of a specific host fungus (e.g., *Tulasnella* spp.) is critical to the germination and development of this species, predominant threats also include factors that have the potential to affect the host fungus' survival or persistence. Threats are listed in order of decreasing level of concern.

### ***Residential Development and Urbanization***

Development associated with urbanization (including housing, infrastructure and other urban development) poses a serious threat to several populations on private property in the Windsor - LaSalle area of Ontario, where several Purple Twayblade occurrences are concentrated within a developing urban area (COSEWIC 2010). In 2008, two sub-populations were believed to be at risk of being lost to housing development (P. Pratt, pers. comm. 2008) and it appears at least one of them (Sandwich West) has been lost since development began. The population at Happy Valley, immediately adjacent to a private home, may be threatened by landscaping and/or by clearing of encroaching vegetation, if it is still existing (COSEWIC 2010). Historically, at least one other sub-population of Purple Twayblade was extirpated when the habitat was destroyed for urban development (COSEWIC 2010).

### ***Alteration of the Natural Disturbance Regime***

Purple Twayblade is found more frequently in open to semi-open habitats, and is a colonizing species of successional and disturbed habitats; populations often decline in size when conditions become fully shaded (Sheviak 1974; Mattrick 2004). Natural disturbances (such as wildfire) and unnatural disturbances (such as grazing, brush cutting) that may have helped to maintain early-successional conditions in the past are no longer as frequent at a landscape scale. Some sites re-surveyed in 2008 were found to be fully shaded with overgrown understory vegetation and no Purple Twayblade observations (Appendix C). Several historical populations that have not been observed for more than 20 years may be extirpated due to succession, which has rendered the habitat unsuitable. In addition, habitat succession is the suspected cause of observed declines at many currently extant populations (COSEWIC 2010). Forest succession is considered the single greatest threat to this species in New England (Mattrick 2004).

### ***Invasive Plants***

Invasive, non-native plant species can impact native species in a variety of ways, from direct competition for resources, displacement, reduction in species diversity and richness (Wilson 1989; Mooney and Cleland 2001) to changes in the fire regime (Brooks et al. 2004). The spread of invasive species has led to a decline in habitat quality for Purple Twayblade (COSEWIC 2010). Invasive plant species were present at four populations (or sub-populations) visited during COSEWIC fieldwork in 2008. Species of concern include Garlic Mustard (*Alliaria petiolata*), European Buckthorn

(*Rhamnus cathartica*), and Scots Pine (*Pinus sylvestris*). The direct effect of these plants on Purple Twayblade is not known. However, research on Garlic Mustard suggests that it may limit native plant growth by interfering with the formation of mycorrhizal associations (Roberts and Anderson 2001). European Buckthorn is known to secrete a compound called emodin, which may have allelopathic<sup>20</sup> 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. However, Purple Twayblade sometimes occurs in areas dominated by other non-native species such as Multiflora Rose (*Rosa multiflora*) and Red Raspberry (*Rubus idaeus* spp. *strigosus*) (G. Waldron, pers. comm. 2008; M. Penskar, pers. comm. 2008). These species, although non-native, may not have direct negative effects on Purple Twayblade growth and survival.

### ***Agricultural Development***

Habitat conversion for agricultural development was historically a threat to Purple Twayblade, and may currently be a threat to some populations. At least three Purple Twayblade populations on private land are situated within the highly developed agricultural landscape of southwestern Ontario. The risk that they may be lost to agricultural development is not known, but it is possible. The extirpation of a population of Purple Twayblade near Arva, Ontario, is believed to have resulted from agricultural expansion that destroyed the habitat (Allen 1989). In addition, intensive agricultural practices may also impact nearby Purple Twayblade populations by reducing availability of pollinators (Rioux Paquette et al. 2013).

### ***Flooding Caused by Beaver Activity***

Although Purple Twayblade is sometimes reported from wetland habitats, such as forested swamps, it appears to favour mesic to moist habitats and may be unable to tolerate prolonged periods of permanent flooding. The Frontenac Provincial Park population, last reported in 2003 at between 400-500 plants, was flooded by a beaver dam the following year. The dam was subsequently removed by park staff, but has since been rebuilt (M. Sly, pers. comm. 2014). As such, this population may be extirpated, as plants have not been observed again, despite annual monitoring.

### ***Invasive Invertebrates***

Invasive, non-native invertebrates may be a threat to Purple Twayblade at some populations, either through direct herbivory, or possibly due to changes in soil composition and structure. For example, leaf damage by exotic slugs has been observed in Purple Twayblade populations near urban areas (G. Waldron, pers. comm. 2008) which could affect plant survival and/or reproductive success. Evidence exists

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<sup>20</sup> A usually negative affect on the growth or development of an organism of one species, caused by a chemical released by an organism of another species.

that non-native earthworms occur in many smaller woodlands and multiple soil types in southern Ontario, and further expansion of their range is possible (Addison 2009). Non-native earthworms can cause significant changes in plant communities, including a loss of herbaceous plant and fungal diversity, and reduction of the duff<sup>21</sup> layer (Muratake 2003; Hale et al. 2006). The direct effects of non-native earthworms on Purple Twayblade are not known.

### ***Herbivory***

Grazing by White-tailed Deer (*Odocoileus virginianus*) may threaten Purple Twayblade in southern Ontario where deer populations are high. It is not known whether White-tailed Deer are affecting Canadian populations of Purple Twayblade because herbivory on Canadian populations has not been directly observed or documented. However, orchids are known to be favoured by deer (Whigham 1990). Deer, rabbits (*Sylvilagus* spp.) and Wild Turkeys (*Meleagris gallopavo*) have all been observed browsing on the species in the United States (Mattrick 2004) and are considered a threat to New England populations.

### ***Herbicide, Fungicide and Pesticide Application***

The effects of herbicides and fungicides on Purple Twayblade have not been documented in the scientific literature, and it is not known whether this is currently a threat to the species. Close observations of the Komoka population in the 1970s suggest that the population may have been extirpated when gramoxone<sup>22</sup> was sprayed onto crops adjacent to the woodland (Allen 1989). The use of these chemicals in proximity to Purple Twayblade populations would likely reduce or destroy populations of soil mycorrhizae.

Similarly, the effects of pesticides on pollinator populations would likely impact Purple Twayblade by reducing the availability of pollinators. A number of factors are suspected to be contributing to the decline in insect pollinator populations globally and in Canada, including loss of habitat and food sources, diseases, viruses, pests, and pesticide exposure (Health Canada 2014). Notably, there is growing evidence to suggest that pesticides, including neonicotinoids, may be having negative effects on pollinator populations due to their toxic properties and persistence in soil and water (van der Sluijs et al. 2013; Cutler et al. 2014).

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<sup>21</sup> Decaying leaves or branches covering a forest floor.

<sup>22</sup> Quick-acting and non-selective herbicide that kills green plant tissue on contact and is one of the most widely used herbicides in the world.

## 5. Population and Distribution Objectives

The population and distribution objectives for Purple Twayblade in Canada are:

- Maintain the abundance and number of extant populations and corresponding sub-populations;
- Where biologically and technically feasible, increase population abundance at extant populations and restore historical populations, and;
- Maintain the approximate distribution of the extant populations and corresponding sub-populations.

Though Purple Twayblade was probably relatively rare, occurring at the northern edge of its range in Canada, it nonetheless was more widespread than it is today as seven of the documented populations are now considered extirpated or historical in Canada. In addition, it is unclear whether Purple Twayblade plants or suitable habitat (or opportunities for habitat restoration) remain at three populations where no plants were detected during the last field visit but the population is currently considered extant. If determined to be feasible, restoration of historical populations through habitat management, as well as habitat improvement at extant populations would be required to meet the population and distribution objectives. Though several techniques exist or are in development, propagation and transplantation is not currently being recommended; rather, recovery will focus on maintaining existing populations and natural expansion/re-establishment of populations through habitat management.

Most extant populations of Purple Twayblade have fewer than 30 plants and it is therefore possible that isolation and fragmentation of populations, and consequently inbreeding depression, contribute to a decline in population viability. Though information on minimum viable population size is lacking, increases to the abundance at these populations (and possibly others) would be required to improve viability and persistence (reducing isolation, fragmentation and the potential for inbreeding depression). If determined to be feasible, to increase the abundance of Purple Twayblade, it will likely be necessary to conduct active habitat management. Locations with extant populations should be prioritized; however, it is possible that historical locations with habitat still intact (i.e., not developed) contain dormant individuals, viable seeds in the seed bank and/or the required mycorrhizal fungus, and therefore opportunities for restoration should be explored. Information on the fungus, longevity of the seed bank or whether Purple Twayblade remains dormant (i.e., underground) like many other orchids is lacking. Additional research into the species' ecology and reproduction is required to determine the feasibility of restoring historical populations and increasing extant population sizes.

Because Purple Twayblade is a colonizing species of early-successional habitats, the location of populations may be somewhat dynamic. However, the species appears to be restricted by the presence and abundance of the specific mycorrhizal fungus required for establishment and growth. Minor changes to the configuration of the Canadian distribution of Purple Twayblade are expected. Maintaining the currently extant

populations in the exact locations in which they are found may not be possible over the long term. Where habitat conditions are improved, and provided that the fungal associate remains present, it is possible that Purple Twayblade populations may increase locally, and colonize or recolonize areas of nearby suitable habitat. Therefore, maintaining the approximate distribution in Ontario and Quebec is considered an appropriate objective for recovery.

## **6.**

## **6. Broad Strategies and General Approaches to Meet Objectives**

### **6.1 Actions Already Completed or Currently Underway**

Within the last decade, many areas where extant populations of Purple Twayblade occur have been secured by public agencies or conservation organizations. Four populations occur on lands now owned and/or managed by the Nature Conservancy of Canada (NCC) or Ontario Parks. At least five previously known populations (or sub-populations) in Ontario are now within the ownership of either the City of Windsor or the Town of LaSalle. This change in ownership has increased the proportion of Purple Twayblade habitat that is under public and/or conservation ownership, and has significantly reduced the likelihood of several populations and/or sub-populations being lost to future development.

A prescribed burn was conducted adjacent to Purple Twayblade habitat in Black Oak Woods, owned by the City of Windsor, in 2007. The populations on lands owned by the City of Windsor are frequently observed and informally monitored by staff at the Ojibway Nature Centre (P. Pratt, pers. comm. 2008).

Property management plans have been developed for the two properties owned by the NCC where Purple Twayblade occurs (or has occurred) (Pelee Island - Shaughnessy Cohen Memorial Savannah and Oxley Poison Sumac Swamp). The management plans address broad issues of conservation management for each property (e.g., invasive species control). During recent fieldwork at Shaughnessy Cohen Memorial Savannah, NCC staff observed invasive species within the habitat of Purple Twayblade (M. McFarlane, pers. comm. 2014). In addition, NCC also acquired a property designated as a nature reserve under Quebec's *Natural Heritage Conservation Act*, though it is located within the perimeter of critical habitat, no Purple Twayblade plants have been found there to date.

A beaver dam that had caused the flooding of Purple Twayblade habitat in Frontenac Provincial Park was removed in 2007, but has subsequently been rebuilt. The area has been monitored annually by the Friends of Frontenac Park, although no Purple Twayblade plants have been observed (M. Sly, pers. comm. 2014).



The population on McDonald Campus of McGill University in Montreal, Quebec is monitored regularly (A. Godbout, pers. comm. 2014). A partial inventory of this population was conducted in 2010 and completed in 2011 (Appendix C). In 2010, a more in-depth study took place where, in addition to the number of individuals, information on habitat, soil composition and spatial configuration of the orchids was collected (Murphy and Idziak 2011).

Recent discoveries of Purple Twayblade within the restoration sites established for the HGP development occurred in 2012 and 2013. These individuals will be subject to long-term habitat protection required under an ESA permit. The habitat is being actively managed to perpetuate tallgrass prairie through selective brush-cutting, invasive species management, and prescribed burns; long-term protection and active management of the tallgrass prairie habitat is expected to help maintain and/or potentially increase Purple Twayblade populations (AMEC 2014).

## 6.2 Strategic Direction for Recovery

**Table 2. Recovery Planning Table**

Threat or Limitation	Priority <sup>23</sup>	Broad Strategy to Recovery	General Description of Research and Management Approaches
All threats	High	Monitor / assess populations	<ul style="list-style-type: none"> <li>• Conduct habitat assessments and regular population monitoring at extant and historical populations where suitable habitat still exists, prioritizing surveys on private properties near urban centres and populations (or sub-populations) that haven't been visited in recent years; update species and habitat mapping.</li> <li>• Identify opportunities for improving or restoring habitat to increase abundance at extant populations and/or re-establish historical populations; prioritize populations (or sub-populations) for habitat management.</li> <li>• Develop and implement standardized methods for accurate population counts and monitoring of Purple Twayblade.</li> <li>• Determine species distribution by conducting surveys in unoccupied suitable habitat across the species' range in Canada.</li> </ul>
Residential development and urbanization; Agricultural development; Alteration of the natural disturbance regime; Flooding caused by Beaver activity; Invasive plants; Invasive invertebrates	High	Conserve and manage habitat	<ul style="list-style-type: none"> <li>• Enable habitat conservation by ensuring municipalities receive results of inventory and monitoring.</li> <li>• Develop and implement habitat management and restoration methods (e.g., invasive species removal, canopy thinning, beaver and/or dam removal, prescribed burning) to maintain and/or restore suitable habitat for Purple Twayblade at extant and historical populations, as appropriate.</li> <li>• Collaborate with land trusts, conservation organizations and public agencies to identify and secure key populations (or sub-populations), prioritizing populations at risk of being destroyed by development, for stewardship, easement or other securement techniques or conservation measures.</li> </ul>
Residential development and urbanization; Agricultural development; Alteration of the natural	Medium	Education and outreach	<ul style="list-style-type: none"> <li>• Identify best land management practices for Purple Twayblade habitat.</li> <li>• Conduct outreach with landowners to communicate the possible impacts of land management practices (e.g., use of herbicides,</li> </ul>

<sup>23</sup> "Priority" reflects the degree to which the broad strategy contributes directly to the recovery of the species or is an essential precursor to an approach that contributes to the recovery of the species.

disturbance regime; Invasive plants; Invasive invertebrates; Herbicide and fungicide application			fungicides and pesticides) and if available, communicate best land management practices for Purple Twayblade habitat.
Knowledge gaps pertaining to effects of habitat management and population restoration	High	Conduct research	<ul style="list-style-type: none"> <li>• Determine the effects of prescribed fire, canopy thinning, and hand pollination on Purple Twayblade populations, and make recommendations on their use as restoration techniques.</li> </ul>
Knowledge gaps pertaining to recruitment and reproduction, herbivory, pollinators and species distribution	Medium	Conduct research	<ul style="list-style-type: none"> <li>• Determine factors influencing the establishment of Purple Twayblade (e.g., dormancy, longevity of seeds in the seed bank, prolonged submersion, pollinator species), including the identity, distribution, abundance and requirements of fungal associates.</li> <li>• Determine factors influencing the establishment of the species at the Morgan Arboretum population to better understand the positive attributes of the largest known population in Canada.</li> <li>• Establish and test a propagation protocol.</li> <li>• Determine level of herbivory at extant populations and its effects on Purple Twayblade populations.</li> </ul>

## **7. Critical Habitat**

### **7.1 Identification of the Species' Critical Habitat**

Section 41 (1)(c) of SARA requires that recovery strategies include an identification of the species' critical habitat, to the extent possible, as well as examples of activities that are likely to result in its destruction. Under SARA, critical habitat is "the habitat that is necessary for the survival or recovery of a listed wildlife species and that is identified as the species' critical habitat in the recovery strategy or in an action plan for the species".

Critical habitat for Purple Twayblade in Canada is identified for ten extant populations in Ontario and Quebec based on best available information as of February 2015 (see Figures 3 and 4, and also Table 3). It is recognized that the critical habitat identified below is insufficient to achieve the population and distribution objectives for the species. A Schedule of Studies (section 7.2; Table 4) has been developed and outlines the activities required for identification of additional critical habitat necessary to support the population and distribution objectives. Additional critical habitat may be added in the future, as new information becomes available.

Critical habitat for Purple Twayblade is based on two criteria: habitat occupancy and habitat suitability.

#### **7.1.1 Habitat Occupancy**

This criterion refers to areas where there is a reasonable degree of certainty of current use by the species.

Habitat is considered occupied when:

- One or more native Purple Twayblade individuals have been observed in any single year since 1995.

Habitat occupancy is based on a timeframe that is consistent with NatureServe's (2002) and Ontario's NHIC threshold for considering populations to be extant versus historical, and allows for inclusion of a number of (sub) populations for which recent population status could not be confirmed during field visits performed in 2008 to support the COSEWIC assessment. The twenty-year window (since 1995) is also based upon the maximum apparent dormancy period reported for other orchids existing in Canada (Light and MacConnail 2006). Although this species has not demonstrated below-ground dormancy to the same extent as other native Canadian orchids (e.g., Nodding Pogonia), the twenty-year time frame was chosen to ensure that all potentially occupied populations are captured.

#### **7.1.2 Habitat Suitability**

Habitat suitability relates to areas possessing a specific set of biophysical attributes that can support individuals of the species in carrying out essential aspects of their life cycle.

Purple Twayblade occurs across a fairly wide range of habitats in southern Ontario and Quebec (COSEWIC 2010). At extant populations in Canada, Purple Twayblade is found in oak woodland and savanna, mixed deciduous forest, shrub-thicket, shrub alvar, forested swamps (deciduous and mixed deciduous-coniferous), tallgrass prairie or coniferous plantations. Despite an apparently wide tolerance to a variety of habitat types, Purple Twayblade is only found in certain locations, owing to its obligate association with soil mycorrhizae (McCormick et al. 2006; 2012). The presence and abundance of the mycorrhizal soil fungus that supplies nutrients to the orchid is critical to the orchid's existence. There is strong evidence that plants in all stages of development require this fungal associate (Whigham, pers. comm. cited in Matlack 2004; McCormick et al. 2004).

Purple Twayblade is found in a variety of canopy conditions. It prefers open to semi-open canopies, and appears to have the ability to colonize areas following disturbance (Sheviak 1974). Nonetheless, it is sometimes present in forests or swamps with dense canopies (Sheviak 1974; White 2008; Homoya 2012).

Canadian populations of Purple Twayblade are found mainly in upland (well-drained, upper slope) habitat, with the exception of two more recently discovered occurrences that are in forested swamps (deciduous and mixed deciduous-coniferous). It can exist in a variety of soil conditions and textures, from sands to silt and clay loams (Sheviak 1974). It is generally found in mildly acidic soils (pH 4.5 to 6.6) but is tolerant of a range of conditions, from strongly acidic to circumneutral soils (Sheviak 1974; Smith 1993; Homoya 2012). The presence of Purple Twayblade may be more dependent upon community successional stage and light levels than on soil pH.

The biophysical attributes of suitable habitat include the characteristics described below:

- Presence of mycorrhizal soil fungus (i.e., *Tulasnella*)
- Variety of canopy conditions, from open (<25%) to closed (>60%); open to semi-open (between 25 and 60%) being the most common
- Variety of moisture conditions, from wetlands to uplands; mesic to xeric<sup>24</sup> being the most common
- Variety of soil types, from sands to silts to clay loams
- Variety of soil pH, from strongly acidic to circumneutral<sup>25</sup>; mildly acidic (pH 4.5 to 6.6) being the most common

Based on the best available information, suitable habitat for the Purple Twayblade is currently defined as the extent of the biophysical attributes where the Purple Twayblade exists in Ontario and Quebec. In addition, a critical function zone of 50 m (radial distance) is applied when the biophysical attributes around a plant extend for less than 50 m.

In Ontario, suitable habitat for the Purple Twayblade is best described using the Ecological Land Classification (ELC) framework for southern Ontario (Lee et al. 1998).

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<sup>24</sup> Dry.

<sup>25</sup> Nearly neutral; having a pH between 6.5 and 7.5.

The ELC framework provides a standardized approach to the interpretation and delineation of dynamic ecosystem boundaries. The ELC approach classifies habitats not only by vegetation community but also considers soil moisture conditions and topography, and as such provides a basis for describing the ecosystem requirements of the habitat for Purple Twayblade. In Ontario, ELC terminology and methods are familiar to many land managers and conservation practitioners who have adopted this tool as the standard approach for habitat classification in Ontario.

Within the ELC system in Ontario, the ecosite level best captures the extent of biophysical attributes required by the species. The ecosite includes the areas occupied by Purple Twayblade and the surrounding areas that provide suitable habitat conditions to carry out essential life process for the species and should allow for natural processes related to population dynamics and reproduction (e.g., dispersal and pollination) to occur.

In Quebec, suitable habitat can be described using the *Cadre écologique de référence du Québec* (CERQ) developed by the Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques (MDDELCC 2015). The CERQ approach to ecosystem classification is very similar to the ELC used in Ontario. Specifically, the CERQ allows for a standardized approach to defining ecosystem boundaries found in Quebec using geology, terrain, surface deposits (e.g., soils), landforms and hydrology. The CERQ system uses up to seven nested levels, four of which are mapped and currently available.

Within the CERQ, the *élément topographiques* level best captures the extent of biophysical attributes required by Purple Twayblade in Quebec. The *élément topographiques* is defined on the same scale as the ecosite under the ELC and includes the areas occupied by Purple Twayblade and the surrounding areas that provide suitable habitat conditions to carry out essential life process for the species and should allow for natural processes related to population dynamics and reproduction (e.g., dispersal and pollination) to occur.

Purple Twayblade is a colonizing orchid that occurs in a wide variety of habitats and whose distribution is likely restricted by the presence of a specific mycorrhizal fungus which it relies upon for successful germination of seeds and survival. With the exception of the immediate area where Purple Twayblade plants are growing and by conducting molecular analysis of DNA, it is not possible to ensure the ELC ecosite or the CERQ *élément topographiques* captures the fungus, about which very little is known regarding its distribution and ecology in Canada. Studies have found that germination of orchid seeds decreases with increasing distance from adult plants, which suggests mycorrhizae exist in proximity to adult plants (McKendrick et al. 2002; Diez 2007; Murphy and Idziak 2011). It is believed the immediate area surrounding Purple Twayblade populations is more likely to contain the appropriate soil mycorrhizal fungus. It is possible that Purple Twayblade populations may increase locally, and colonize or recolonize areas of nearby suitable habitat within the ELC ecosite or the CERQ *élément topographiques*.

The 50 m radial distance is considered a minimum 'critical function zone', or the threshold habitat fragment size required for maintaining constituent microhabitat properties for a species (e.g., essential light, moisture, humidity levels necessary for survival). At present, it is not clear at what exact distance physical and/or biological processes begin to negatively affect Purple Twayblade, and this distance is likely to depend on local habitat characteristics. Studies on micro-environmental gradients at habitat edges, i.e., light, temperature, litter moisture (Matlack 1993), and of edge effects on plants in mixed hardwood forests, as evidenced by changes in plant community structure and composition (Fraver 1994), have shown that edge effects could be detected up to 50 m into habitat fragments, although other studies show that the magnitude and distance of edge effects will vary depending on the structure and composition of adjacent habitat types (Harper et al. 2005). A 50 m radial distance from a Purple Twayblade plant was chosen to ensure that microhabitat properties are maintained as part of the identification of critical habitat. The area within the critical function zone may include both suitable and unsuitable habitat as Purple Twayblade may be found near the transition zone between suitable and unsuitable habitat (e.g., within small forest openings, or along woodland edges). As new information on species' habitat requirements and site-specific characteristics, such as hydrology, become available, distances may be refined.

Paved areas or built-up features such as buildings do not possess the biophysical attributes of suitable habitat or assist in the maintenance of natural processes.

### 7.1.3 Application of the Purple Twayblade Critical Habitat Criteria

Critical habitat for Purple Twayblade is identified as the extent of suitable habitat (section 7.1.2) where the habitat occupancy criteria is met (section 7.1.1). In cases where the suitable habitat extends for less than 50 m around a Purple Twayblade, a critical function zone capturing an area within a radial distance of 50 m is also included as critical habitat.

In Ontario, as noted above, suitable habitat for Purple Twayblade is most appropriately identified at the ELC ecosite level. At the present time, ecosite descriptions and boundaries are not available to support the identification of critical habitat for all populations in Ontario. In the interim, the ELC community series level is identified as the area within which critical habitat is found. In Ontario, critical habitat is located within these boundaries where the biophysical attributes described in section 7.1.2 are found and where the occupancy criterion is met (section 7.1.1). When ecosite boundaries are determined, the identification of critical habitat will be updated.

In Quebec, as noted above, suitable habitat for Purple Twayblade is most appropriately identified at the *élément topographiques* level of the CERQ. At the present time, the *élément topographiques* descriptions and boundaries are not available to support the identification of critical habitat for the population in Quebec. In the interim, the ecological district level under the CERQ combined with the forest ecosystem layer of the *Répertoire des projets d'identification des milieux naturels d'intérêt du Québec méridional* (EC and MDDELCC 2015) is used to define the area within which critical habitat is found. In

Quebec, critical habitat is located within these boundaries where the biophysical attributes are found (section 7.1.2) and where the occupancy criterion is met (section 7.1.1). When *élément topographiques* boundaries are determined, the identification of critical habitat will be updated.

Application of the critical habitat criteria to the best available information identified critical habitat for ten extant populations of Purple Twayblade in Canada (Figure 3 and Figure 4; see also Table 3), totaling up to 421 ha<sup>26</sup>. The critical habitat identified is considered a partial identification of critical habitat and is insufficient to meet the population and distribution objectives. As it is unknown whether Purple Twayblade (or its fungal associate) can survive prolonged submersion and thus whether suitable habitat is still present, critical habitat is not currently identified for the Frontenac Provincial Park population. Further, the feasibility of restoring historical populations needs to be investigated at historically occupied sites. A schedule of studies (section 7.2) has been developed to provide the information necessary to complete the identification of critical habitat that will be sufficient to meet the population and distribution objectives.

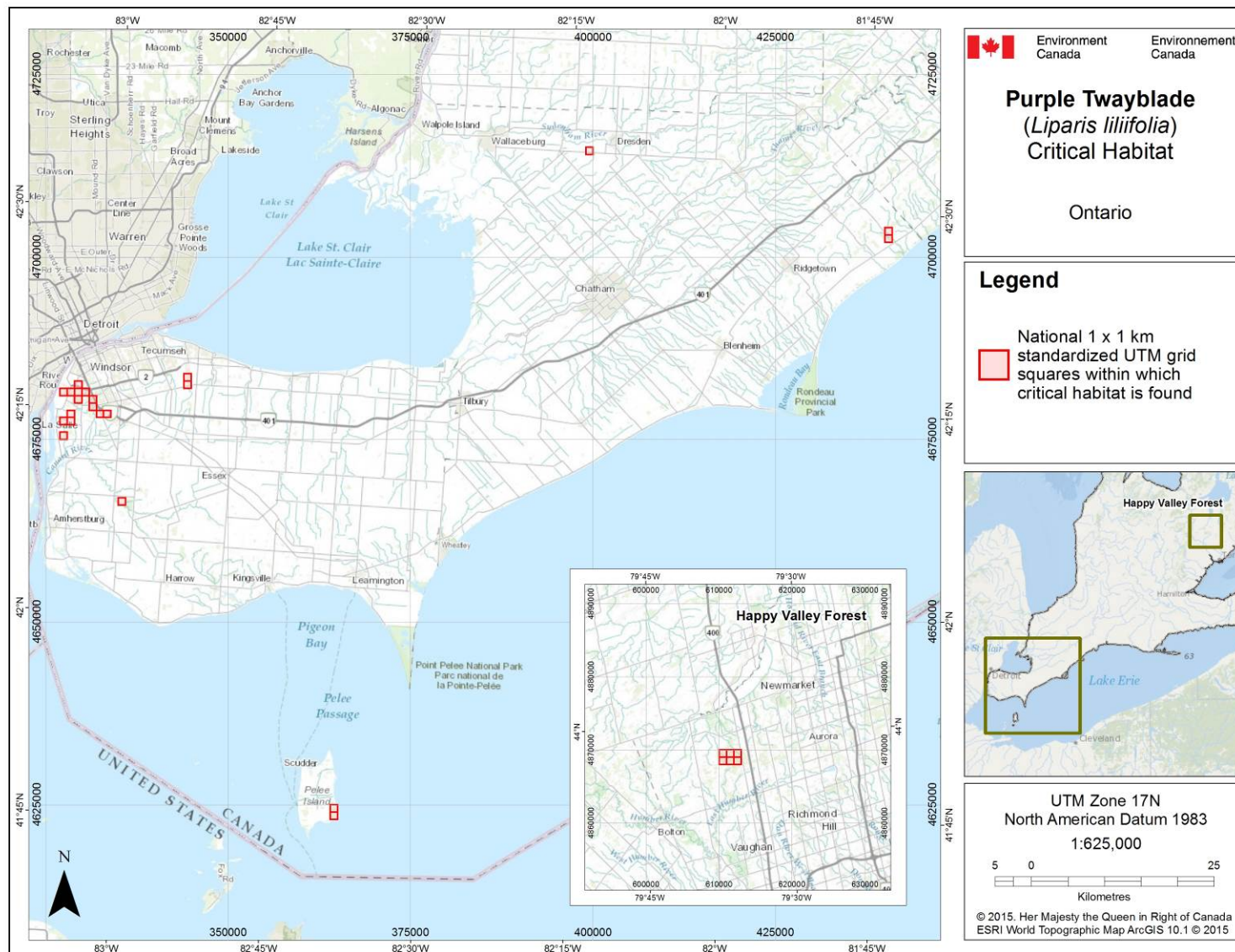
While no Purple Twayblade plants were observed within the *Endangered Species Act*, 2007 permit boundary of the HGP, the portion of one sub-population within the Ojibway Prairie Complex population (Final Restoration Site #21 – Appendix C) that is partially within this permit boundary is not currently identified as critical habitat. Upon completion of the HGP and as restoration sites for other species at risk plants have become established, critical habitat will be reviewed and additional critical habitat may be identified.

Critical habitat for Purple Twayblade is presented using 1 x 1 km UTM grid squares (Table 3). The UTM grid squares presented in Figure 3 and Figure 4 are part of a standardized grid system that indicates the general geographic areas containing critical habitat, which can be used for land use planning and/or environmental assessment purposes. In addition to providing these benefits, the 1 x 1 km UTM grid respects provincial data-sharing agreements in Ontario. Critical habitat within each grid square occurs where the description of habitat occupancy (section 7.1.1) and habitat suitability (section 7.1.2) are met. More detailed information on critical habitat may be requested on a need-to-know basis by contacting Environment Canada – Canadian Wildlife Service at [ec.planificationduretablissement-recoveryplanning.ec@canada.ca](mailto:ec.planificationduretablissement-recoveryplanning.ec@canada.ca).

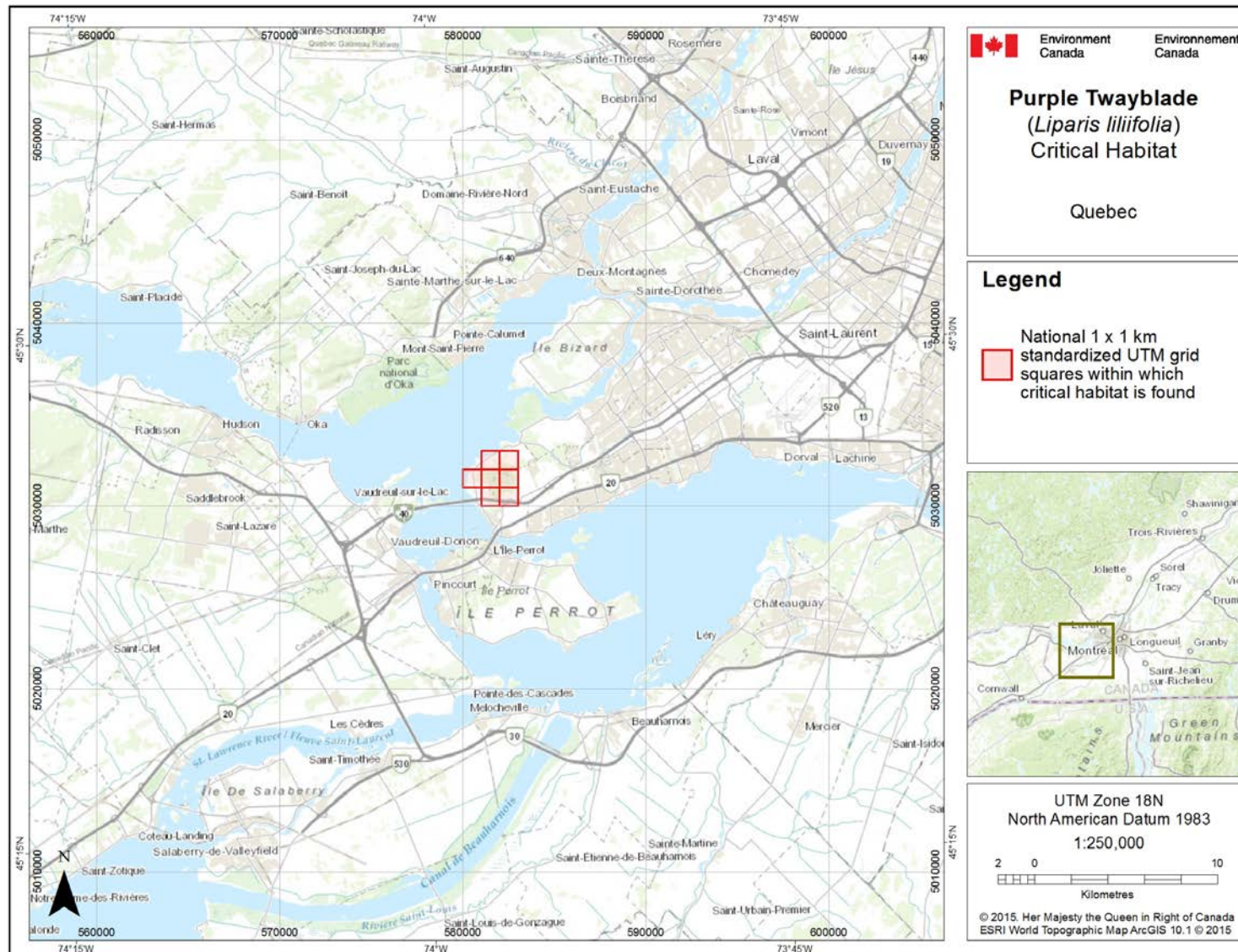
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<sup>26</sup> This is the maximum extent of critical habitat based on habitat boundaries that can be delineated from high resolution aerial photography (comparable to ELC, Community Series) and/or a 50m radial distance around the Purple Twayblade. Actual critical habitat occurs only in those areas described in section 7.1 and therefore the actual area could be less than this and would require field verification to determine the precise amount.





**Figure 3. Grid squares that contain critical habitat for the Purple Twayblade in Ontario.** Critical habitat for the Purple Twayblade occurs within these 1 x 1 km standardized UTM grid squares (red shaded outline), where the description of habitat occupancy (section 7.1.1) and habitat suitability (section 7.1.2) are met. The inset map shows the critical habitat at the Happy Valley Forest population.



**Figure 4. Grid squares that contain critical habitat for the Purple Twayblade in Quebec.** Critical habitat for the Purple Twayblade occurs within these 1 x 1 km standardized UTM grid squares (red shaded outline), where the description of habitat occupancy (section 7.1.1) and habitat suitability (section 7.1.2) are met



**Table 3. Grid squares that contain critical habitat for the Purple Twayblade in Canada.**  
Critical habitat for the Purple Twayblade occurs within these 1 x 1 km standardized UTM grid squares where the description of habitat occupancy in section 7.1.1 and habitat suitability (section 7.1.2) are met.

Population	Sub-population	1 x 1 km standardized UTM grid square ID <sup>1</sup>	Province	UTM Grid Square Coordinates <sup>2</sup>		Land Tenure <sup>3</sup>
				Easting	Northing	
1. Pelee Island - Shaunessy Cohen Nature Reserve		17TLG6244	Ontario	364000	4624000	Non-federal Land
		17TLG6243	Ontario	364000	4623000	Non-federal Land
2. Ojibway Prairie Complex and Area	Chappus Street Restoration Block	17TLG2892	Ontario	329000	4682000	Non-federal Land
		17TLG3801	Ontario	330000	4681000	Non-federal Land
	Chappus Street Restoration Block/Tallgrass Heritage Park and Ojibway Prairie	17TLG2891	Ontario	329000	4681000	Non-federal Land
	Tallgrass Heritage Park and Ojibway Prairie	17TLG2890	Ontario	329000	4680000	Non-federal Land
	Black Oak Woods	17TLG2881	Ontario	328000	4681000	Non-federal Land
		17TLG2871	Ontario	327000	4681000	Non-federal Land
	Spring Garden ANSI	17TLG3810	Ontario	331000	4680000	Non-federal Land
		17TLG3719	Ontario	331000	4679000	Non-federal Land
	Final Restoration Site #21	17TLG3738	Ontario	333000	4678000	Non-federal Land
		17TLG3728	Ontario	332000	4678000	Non-federal Land
3. Reaume Street Prairie	Reaume Street Prairie	17TLG2788	Ontario	328000	4678000	Non-federal Land
	Reaume Street Prairie/Town of LaSalle Candidate Natural Heritage Area TC5/M1	17TLG2787	Ontario	328000	4677000	Non-federal Land
	Town of LaSalle Candidate Natural Heritage Area TC5/M1	17TLG2777	Ontario	327000	4677000	Non-federal Land

Population	Sub-population	1 x 1 km standardized UTM grid square ID <sup>1</sup>	Province	UTM Grid Square Coordinates <sup>2</sup>		Land Tenure <sup>3</sup>
				Easting	Northing	
4. Town of LaSalle Candidate Natural Heritage Area CH-M11		17TLG2775	Ontario	327000	4675000	Non-federal Land
5. McAuliffe Woods Conservation Area		17TLG4843	Ontario	344000	4683000	Non-federal Land
		17TLG4842	Ontario	344000	4682000	Non-federal Land
6. Canard River - Mitchell Property		17TLG3656	Ontario	335000	4666000	Non-federal Land
9. Deyo's Woods		17TLH9194	Ontario	399000	4714000	Non-federal Land
10. Clear Creek		17TMH4003	Ontario	440000	4703000	Non-federal Land
		17TMH4002	Ontario	440000	4702000	Non-federal Land
13. Happy Valley Forest		17TPJ1609	Ontario	610000	4869000	Non-federal Land
		17TPJ1619	Ontario	611000	4869000	Non-federal Land
		17TPJ1629	Ontario	612000	4869000	Non-federal Land
		17TPJ1608	Ontario	610000	4868000	Non-federal Land
		17TPJ1618	Ontario	611000	4868000	Non-federal Land
		17TPJ1628	Ontario	612000	4868000	Non-federal Land
15. Morgan Arboretum, Macdonald Campus, McGill University		18TWR8301	Quebec	580000	5031000	Non-federal Land
		18TWR8310	Quebec	581000	5030000	Non-federal Land
		18TWR8311	Quebec	581000	5031000	Non-federal Land
		18TWR8312	Quebec	581000	5032000	Non-federal Land
		18TWR8320	Quebec	582000	5030000	Non-federal Land
		18TWR8321	Quebec	582000	5031000	Non-federal Land
		18TWR8322	Quebec	582000	5032000	Non-federal Land
Total = 35 grid squares						

<sup>1</sup>Based on the standard UTM Military Grid Reference System (see <http://www.nrcan.gc.ca/earth-sciences/geography-boundary/mapping/topographic-mapping/10098>), where the first 2 digits and letter represent the UTM Zone, the following 2 letters indicate the 100 x 100 km Standardized UTM grid followed by 2 digits to represent the 10 x 10 km Standardized UTM grid. The last 2 digits represent the 1 x 1 km Standardized UTM grid containing all or a portion of the critical habitat unit. This unique alphanumeric code is based on the methodology produced from the Breeding Bird Atlases of Canada (See <http://www.bsc-eoc.org/> for more information on breeding bird atlases).

<sup>2</sup>The listed coordinates are a cartographic representation of where critical habitat can be found, presented as the southwest corner of the 1 x 1 km Standardized UTM grid square containing all or a portion of the critical habitat unit. The coordinates are provided as a general location only.

<sup>3</sup>Land tenure is provided as an approximation of the types of land ownership that exist at the critical habitat units and should be used for guidance purposes only. Accurate land tenure will require cross referencing critical habitat boundaries with surveyed land parcel information.

## 7.2 Schedule of Studies to Identify Critical Habitat

**Table 4. Schedule of Studies to Identify Critical Habitat**

Description of Activity	Rationale	Timeline
Determine population status and presence of suitable habitat (including presence of mycorrhizal fungal associate) at Frontenac Provincial Park.	It is unclear if Purple Twayblade plants and/or the mycorrhizal fungal associate can withstand prolonged submersion underwater. Population status and presence of suitable habitat is required to allow for additional critical habitat to be identified.	2016-2023
Investigate the feasibility of restoring the species at historically occupied sites by determining whether suitable habitat or opportunities for habitat restoration exist and are likely to be successful. Assessing feasibility will likely require a further understanding of Purple Twayblade ecology (e.g., dormancy and longevity of seed bank) to determine if and which habitat restoration and management techniques would be successful.	Though Purple Twayblade was probably relatively rare, it nonetheless was more widespread than today.  If restoration is determined to be feasible and is proven successful and additional habitat becomes occupied and suitable; identify additional critical habitat.	2016-2023

## 7.3 Activities Likely to Result in the Destruction of Critical Habitat

Understanding what constitutes destruction of critical habitat is necessary for the protection and management of critical habitat. Destruction is determined on a case by case basis. Destruction would result if part of the critical habitat was degraded, either permanently or temporarily, such that it would not serve its function when needed by the species. Destruction may result from a single activity or multiple activities at one point in time or from the cumulative effects of one or more activities over time. It should be noted that not all activities that occur in or near critical habitat are likely to cause its destruction. Activities described in Table 5 are examples of those likely to cause destruction of critical habitat for the species; however, destructive activities are not necessarily limited to those listed.

Recognizing that Purple Twayblade is a colonizing species that is able to establish following disturbance, activities such as removal of vegetation and livestock grazing may have the potential to contribute to the future supply of critical habitat, given proper management.

**Table 5. Activities Likely to Result in the Destruction of Purple Twayblade's Critical Habitat**

Description of Activity	Description of Effect in Relation to Function Loss	Details of Effect
Construction of houses, other structures or roads, including removal of soils (e.g., residential or industrial development)	Construction converts habitat and results in the direct loss of critical habitat upon which the species relies for basic survival, successful seed germination and seedling establishment. Direct removal of soil/substrate would render the habitat unsuitable for Purple Twayblade by removing the biophysical attributes required by the species.	<p>When this activity occurs within the bounds of critical habitat, at any time of year, the effects will be direct, and is certain to result in the permanent destruction of critical habitat. There are no possible thresholds for this activity.</p> <p>Use restricted to the surface of existing roadways/access roads and recreational trails would not result in the destruction of critical habitat.</p>
Conversion of land to agriculture, including the removal of vegetation and/or ploughing of soils	Removal of vegetation converts habitat and results in the direct loss of critical habitat upon which the species relies for basic survival, successful seed germination and seedling establishment. Ploughing of soil/substrate would render the habitat unsuitable for Purple Twayblade by disrupting the biophysical attributes (especially soil mycorrhizae) required by the species.	When this activity occurs within the bounds of critical habitat, at any time of year, the effects will be direct, and it is certain to result in the permanent destruction of critical habitat. There are no possible thresholds for this activity.
Introduction of exotic species, especially plants or invertebrates, such as exotic slugs and non-native earthworms (e.g., introduction of non-native plant seeds, plants, foreign soil or gravel) can be unintentionally introduced from activities such as composting or dumping of garden waste, ATV use and livestock grazing.	Exotic species may outcompete Purple Twayblade, and/or result in physical and chemical changes to habitat such that it is no longer suitable for the species.	When this activity occurs within or adjacent to critical habitat, at any time of year, the effects may be direct and/or cumulative. The introduction of an invasive species can lead to gradual destruction of critical habitat over time (i.e., cumulative impacts).
Application of herbicides or fungicides	Herbicides and fungicides may potentially destroy or deplete the soil fungi upon which the species depends for germination and growth, making the habitat no longer suitable for Purple Twayblade.	When this activity occurs within critical habitat areas, its effects will be direct and cumulative, and it is considered likely to cause destruction of critical habitat because it may compromise the soil mycorrhizae upon which the species depends. If it occurs adjacent to a critical habitat area, it may cause destruction if the chemical drifts into critical habitat areas, but the gradient over which effects may occur is not known and is likely dependent upon a number of factors (e.g., type of herbicide/fungicide used, concentration of formula and weather). Until further information is available, this activity is considered to be detrimental at all times of the year. There is no additional information to inform the development of thresholds.

Activities that cause permanent flooding of critical habitat (e.g., impoundment structures, road construction)	Prolonged submersion underwater may affect the survivability of Purple Twayblade plants and the mycorrhizal fungal associate that they rely upon for germination, seedling establishment and growth.	When this activity occurs within critical habitat areas, the effects will be direct and cumulative, and it is considered likely to cause destruction of critical habitat because it may compromise the soil mycorrhizae upon which the species depends.
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## 8. Measuring Progress

The performance indicators presented below provide a way to define and measure progress toward achieving the population and distribution objectives.

Every five years, success of recovery strategy implementation will be measured against the following performance indicators:

- the abundance and number of extant populations and corresponding sub-populations has been maintained;
- population abundance has been increased and historical populations have been restored, where biologically and technically feasible and;
- the approximate distribution of the extant populations and corresponding sub-populations has been maintained.

## 9. Statement on Action Plans

One or more action plans for Purple Twayblade in Canada will be posted on the Species at Risk Public Registry by December 2023.

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## Appendix A: Effects on the Environment and Other Species

A strategic environmental assessment (SEA) is conducted on all SARA recovery planning documents, in accordance with the [\*Cabinet Directive on the Environmental Assessment of Policy, Plan and Program Proposals\*](#)<sup>27</sup>. The purpose of a SEA is to incorporate environmental considerations into the development of public policies, plans, and program proposals to support environmentally sound decision-making and to evaluate whether the outcomes of a recovery planning document could affect any component of the environment or any of the [\*Federal Sustainable Development Strategy\*](#)'s<sup>28</sup> (FSDS) goals and targets.

Recovery planning is intended to benefit species at risk and biodiversity in general. However, it is recognized that strategies may also inadvertently lead to environmental effects beyond the intended benefits. The planning process based on national guidelines directly incorporates consideration of all environmental effects, with a particular focus on possible impacts upon non-target species or habitats. The results of the SEA are incorporated directly into the strategy itself, but are also summarized below in this statement.

In general, protecting the habitat of Purple Twayblade and the ecosystems within which it is found will benefit many other species and ecosystem functions within southern Ontario and western Quebec. The species exists in critically imperiled oak savanna and tallgrass community types in Ontario. Several Purple Twayblade populations in the Windsor - Essex area occur within larger natural areas known to contain other at-risk prairie-savanna plants (e.g., Colicroot (*Aletris farinosa*), Dense Blazing Star (*Liatris spicata*), and Slender Bush-clover (*Lespedeza virginica*)), and many other significant flora and fauna. Other occupied habitats are more common in Canada and contain fewer species at risk, although their conservation will benefit a number of natural areas, ecosystems and species.

The potential for this recovery strategy to inadvertently lead to adverse effects on other species was considered. Some management activities, such as prescribed burns and selective thinning of the forest canopy, have the potential to harm some species, at least in the short term. The maintenance of open conditions within forested settings may not benefit shade-tolerant and/or some forest interior species. The ecological risks of such management activities will be considered before they are undertaken, in order to avoid or mitigate any negative effects. The SEA concluded that overall this strategy will benefit the environment and will not entail significant adverse effects.

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<sup>27</sup> <http://www.ceaa.gc.ca/default.asp?lang=En&n=B3186435-1>

<sup>28</sup> [www.ec.gc.ca/dd-sd/default.asp?lang=En&n=F93CD795-1](http://www.ec.gc.ca/dd-sd/default.asp?lang=En&n=F93CD795-1)

## Appendix B: Subnational Conservation Ranks of Purple Twayblade in the United States

### List and description of various conservation status ranks for Purple Twayblade in the United States (NatureServe 2014)

	Global (G) Rank	National (N) Rank	Sub-national (S) Rank
<b>Purple Twayblade</b> ( <i>Liparis liliifolia</i> )	G5 (secure)	N5 (demonstrably widespread, abundant, and secure)	Alabama (S1) Arkansas (SNR) Connecticut (S1) Delaware (S2) District of Columbia (SNR) Georgia (S3) Illinois (S3S4) Indiana (SNR) Iowa (S3) Kentucky (S4) Maryland (S2S3) Massachusetts (S2) Michigan (S3) Minnesota (SNR) Mississippi (SNR) Missouri (SNR) New Hampshire (SX) New Jersey (S3S4) New York (S1) North Carolina (S3) Ohio (SNR) Oklahoma (S1) Pennsylvania (SNR) Rhode Island (S1) South Carolina (S1) Tennessee (SNR) Vermont (S1) Virginia (S5) West Virginia (S5) Wisconsin (SNR)

Rank Definitions (NatureServe 2014)

S1: Critically Imperilled - At very high risk of extirpation in the jurisdiction (i.e., N - nation, or S -state/province) due to very restricted range, very few populations or occurrences, very steep declines, severe threats, or other factors.

S2: Imperilled - At high risk of extirpation in the jurisdiction due to restricted range, few populations or occurrences, steep declines, severe threats, or other factors.

S3: Vulnerable - At moderate risk of extirpation in the jurisdiction due to a fairly restricted range, relatively few populations or occurrences, recent and widespread declines, threats or other factors.

S4: Apparently Secure – At a fairly low risk of extirpation in the jurisdiction 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.

G5/N5/S5: Secure - At very low risk of extinction or elimination due to a very extensive range, abundant populations or occurrences, and little to no concern from declines or threats.

SX: Extirpated - Adequate surveys by one or more experienced observers at times and under conditions appropriate for the species at the occurrence location, or other persuasive evidence, indicate that the species no longer exists there or that the habitat or environment of the occurrence has been destroyed to such an extent that it can no longer support the species.

SNR: Unranked – National or subnational conservation status not yet assessed.

## Appendix C: Populations of Purple Twayblade in Canada

Population	Sub-population	Location	Last Observed	Status	Number of Plants Observed (Year)
1. Pelee Island - Shaunessy Cohen Nature Reserve		Township of Pelee, ON	2010	E <sup>29</sup>	21 (2003); 27 (2008); 26 (2010)
2. Ojibway Prairie Complex and Area	a <sup>30</sup> . Tallgrass Heritage Park and Ojibway Prairie	City of Windsor, ON	2008	E	6 (2008)
	b. Black Oak Woods		2008		29 (2008)
	c. Spring Garden ANSI <sup>31</sup>		2008		4 (2008)
	d. LaSalle Woods	Town of LaSalle, ON	1979		0 (2008)
	e. Sandwich West		2002		2-4 (2002)
	f. Chappus Street Restoration Block	City of Windsor, ON	2013		42 (2013)
	g. Final Restoration Site #21	Town of LaSalle, ON	2013		2 (2013)
	h. Windsor (behind Health Lab)	City of Windsor, ON	1969		>70 (1969)
3. Reaume Street Prairie	a. Reaume Street Prairie	Town of LaSalle, ON	1997	E	0 (2008)
	b. Town of LaSalle Candidate Natural Heritage Area TC5/M1 <sup>32</sup>		2008		20 (2008)

<sup>29</sup> Extant (E): A population which is considered to be still in existence, i.e., not destroyed or lost (extirpated).

<sup>30</sup> While the Ojibway Prairie Complex population is considered extant, the sub-population 2d - LaSalle Woods is considered historical (i.e., not confirmed for >20 years) and the sub-populations 2e – Sandwich West and 2h – Windsor (behind Health Lab) are considered extirpated as the habitat has been lost to urban development.

<sup>31</sup> Area of Natural and Scientific Interest (ANSI).

<sup>32</sup> This identifier and the one below refer to unique identifiers given to each site assessed in a natural heritage study for the Town of LaSalle (Waldron et al. 2011).



Population	Sub-population	Location	Last Observed	Status	Number of Plants Observed (Year)
4. Town of LaSalle Candidate Natural Heritage Area CH3-M11		Town of LaSalle, ON	2008	E	14 (2008)
5. McAuliffe Woods Conservation Area		Town of Tecumseh, ON	2009	E	~40 (2009)
6. Canard River – Mitchell Property		Town of Amherstburg, ON	2008	E	1 (2008)
7. Oxley Poison Sumac Swamp		Town of Essex, ON	1986	H <sup>33</sup>	0 (2005, 2006); Not visited in 2008
8. Cedar Creek	a. Cedar Creek - North	Town of Kingsville, ON	1985	H	0 (2008)
	b. Cedar Creek - South		1982		Not visited in 2008
9. Deyo's Woods		Municipality of Chatham-Kent, ON	1997	E	0 (2008)
10. Clear Creek		Municipality of Chatham-Kent, ON	2008	E	253 (2001); 33+ (partial count, 2008)
11. Lakeshore Woods, near New Glasgow		Municipality of West Elgin, ON	1986	H	Not visited in 2008
12. West Lorne, Allan Craig Woods		Municipality of West Elgin, ON	1985	H	Not visited in 2008
13. Happy Valley Forest		Township of King, ON	2000	E	"a few" (2000) 0 (2001, 2008)
14. Frontenac Provincial Park		Township of South Frontenac, ON	2005	E	400-500 (2003 estimate); 55 (2004); 3 (2005); 0 (2008)
15. Morgan Arboretum, Macdonald Campus, McGill University		Montreal, QC	2011	E	186 (2007); 473 (partial count, 2010); 516 (partial count, 2011)

<sup>33</sup> Historical (H): In the absence of known disturbance and with the habitat still extant, H is generally recommended for occurrences that have not been reconfirmed for 20 or more years.

Population	Sub-population	Location	Last Observed	Status	Number of Plants Observed (Year)
16. Komoka		Municipality of Middlesex Centre, ON	1971	X <sup>34</sup>	Extirpated
17. Arva		Municipality of Middlesex Centre, ON	1950s	X	Extirpated; habitat destroyed
18. Fort Erie		Town of Fort Erie, ON	1864	X	Extirpated

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<sup>34</sup> Extirpated (X): A population which was previously known to occur (i.e., for which there is historical record), but that no longer exists.