



Survey Protocols *for the* Rusty Patched Bumble Bee (*Bombus affinis*)

Version 2.2

April 12, 2019



Photograph by Susan Day

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Survey Protocol Guidance Development

A team of USFWS biologists developed survey protocols for *B. affinis* that are effective in meeting survey objectives and unlikely to negatively impact the species. We obtained informal peer review by bumble bee experts including biologists from local, State, and Federal agencies; scientific and academic institutions; and conservation organizations. We considered the best available information for all aspects of the guidance and will continue to work with surveyors to collect additional data on the distribution and ecology of *Bombus affinis*. This guidance document is subject to continual improvement and modification.

Acknowledgements

We would like to thank the following people for assistance with reviewing earlier drafts of this document (in alphabetical order): Michelle Boon, University of Minnesota; Crystal Boyd, Minnesota Department of Natural Resources; Dan Cariveau, University of Minnesota; Susan Carpenter, University of Wisconsin-Madison Arboretum; Sheila Colla, York University; David Cuthrell, Michigan Natural Features Inventory; Sam Droege, U.S. Geological Survey; Elaine Evans, University of Minnesota; Rich Hatfield, Xerces Society for Invertebrate Conservation; Robert Jean, Environmental Solutions & Innovations, Inc.; Jay Watson, Wisconsin Department of Natural Resources; and Amy Wolf, University of Wisconsin-Green Bay.

Introduction and Purpose

Once widespread and abundant, the rusty patched bumble bee (*Bombus affinis*) has undergone precipitous declines and was listed as an endangered species under the Endangered Species Act on January 11, 2016 (FR 50 CFR 17 3816 January 11, 2017). Just 20 years ago, sightings of *B. affinis* were common, and it was considered relatively abundant across 28 states, the District of Columbia and two Canadian provinces (USFWS 2016, p.3). Since then, it has experienced a swift and dramatic decline in abundance and distribution of approximately 90 percent or more. We are aware of records of *B. affinis* in isolated places within 13 states and 1 province since 2000 (FR 50 CFR 17 3816 January 11, 2017). The species' distribution has declined across its range in the U.S., for example, since 2007; point records occur only in 10 states and 66 counties in the U.S. (USFWS unpublished geodatabase, February 22, 2018). Similar declines have occurred in Canada where it was listed as Endangered on Schedule 1 of the Species at Risk Act in 2012 (Environment and Climate Change Canada 2016, p. iv).

While we recognize the great importance of conducting surveys for bees (and other insects), we need to be particularly cautious in areas that may contain *B. affinis*. The rusty patched bumble bee is so imperiled that every remaining population is important for the continued existence of the species. We must carefully consider the benefits of bee (and other insect) survey versus their potential to negatively impact the rusty patched bumble bee. Therefore, we recommend non-lethal protocols in areas where we may encounter the species (*e.g.*, areas within **High Potential** and

Primary Dispersal Zones where the occupancy is uncertain, see definitions below and online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>).

Survey effort throughout the historic range of *B. affinis* has not been systematic and the occupancy of some sites is uncertain. For these reasons the Service recommends non-lethal survey protocols in areas where there is a relatively high potential of encountering the species and in areas where there is a low, but still some, potential of encountering the species. We have developed a habitat-dispersal distance model (the **Habitat Connectivity Model** is described online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>) that identifies geographic areas to focus survey effort and where to be cautious regarding potential take associated with surveys.

The objectives of the survey protocols are to: (1) Find and document new *B. affinis* locations; (2) Determine if *B. affinis* are still extant at previously documented locations; (3) Monitor bumble bee populations to determine long-term population trends, relative abundance and species richness; and (4) Provide protocol recommendations for areas locations we believe are unoccupied by *B. affinis*.

Because *B. affinis* surveys can result in take (by capture), such surveys should only be conducted by a qualified biologist¹. Generally, a recovery permit for *B. affinis* authorizes the capture of bees for identification and handling of bees for photography. Following this guidance will meet standard USFWS requirements for conducting surveys and monitoring under a federal recovery/scientific permit under section 10(a)(1)(B) of the ESA. However, surveyors also need to ensure they meet all applicable state permitting and reporting requirements. For further information about obtaining a federal permit and surveyor qualifications please see **Obtaining a permit under the Endangered Species Act and Frequently Asked Questions** available online at www.fws.gov/midwest/endangered/insects/rpbb/surveyors.html.

This survey protocol provides the U.S. Fish and Wildlife Service's (USFWS) recommended guidance on survey methodology and outlines additional reporting requirements for permittees. Future changes to this guidance may occur and will be posted on the USFWS rusty patched bumble bee guidance website (www.fws.gov/midwest/endangered/insects/rpbb/surveyors.html) by February 28th of each year. Before conducting surveys, please check the website to ensure use of the most current version of this document. Contact the FWS Field Office near you (<http://www.fws.gov/offices/>) if you are interested in collecting data that is not discussed in this document.

¹ A qualified biologist is an individual who holds a USFWS Recovery Permit (Federal Fish and Wildlife 10(a)(1)(A) Permit) for *B. affinis* in the state/region in which they are surveying and/or has been authorized by the appropriate state agency to capture and handle *B. affinis*. See **Obtaining a permit under the Endangered Species Act and FAQs** available online at www.fws.gov/midwest/endangered/insects/rpbb/surveyors.html.

Definition of Terms used in this Document

For the purposes of this document, a **site** is defined as a record (observation point) and the surrounding area that would typically provide the foraging area for one colony. Studies of other bumble bee species typically exhibit foraging distances of less than 0.6 mi (1 km) from their nesting sites (Knight et al. 2005, p. 1816; Wolf and Moritz 2008, p. 422; Dramstad 1996, pp. 163-182; Osborne et al. 1999, pp. 524-526; Rao and Strange 2012, pp. 909-911). There may be one or more sites within any of the zones described below.

- **High Potential Zones** are based on the habitat connectivity model described in detail online (see **Habitat Connectivity Model** at <https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>). These High Potential Zones contain **extant sites** (see definition below) and the surrounding area considered to have highest potential for the species to be present, as generated from the model. These zones are not of uniform size and have discrete boundaries that can be used to determine where non-lethal surveys or scientific recovery permits may be recommended (Fig.1 and <https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>). The zones range from about 1 to 2 miles (1.6 to 3.2 km) from extant sites. For recommendations regarding protocols and scientific recovery permits in High Potential Zones, see Table 1.
- **Extant Sites** are defined as sites where *B. affinis* has been documented in 2007 or later, unless surveyed sufficiently to be considered unoccupied. Ideally, extant sites will have multiple years with records of the species. If no or little effort has been spent attempting to relocate the species at the site, then additional surveys are needed before considering the site to be unoccupied.
- **Low Potential Zones** include: **Uncertain Zones** (defined below and online at <http://www.fws.gov/midwest/endangered/insects/rpbb/rpbbmap.html>) and **Primary Dispersal Zones** surrounding **High Potential Zones** (Fig.1). For recommendations regarding protocols and scientific recovery permits in Low Potential Zones, see Table 1.
- **Primary Dispersal Zones** are based on the habitat connectivity model described in detail online (see **Habitat Connectivity Model** online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>). The model used to create the Primary Dispersal Zones considers the maximum dispersal potential from known sites and across the range. These zones, although not of uniform size, have discrete boundaries that can be used to determine where non-lethal surveys are recommended and where a scientific recovery permit for surveys might be recommended (Fig.1).
- **Uncertain Zones** have records of the *B. affinis* in 2000 to 2006 out to and including the surrounding dispersal area, but with fewer than 3 years of negative survey data with sufficient effort² since the last known record. If no or little effort has been spent attempting

² Sufficient effort would consist of four approximately equally spaced sampling periods during the sampling season (early June to mid-August); one-person hour of search time per three acres of suitable high quality habitat (defined below) using non-lethal netting techniques. This document provides further details on methods, techniques, and best practices and is subject to continual improvement and modification.

to relocate the species at the site, then additional surveys are needed before considering the site to be unoccupied. Areas remain uncertain until they have either (1) a positive detection, when the site would be considered extant or (2) have at least 3 years of negative survey data (*i.e.*, using the Project Review Protocol or equivalent effort), when they would be considered unoccupied.

- **Unoccupied Zones** are areas where the likelihood of encountering *B. affinis* is so low that the area is considered unoccupied and includes: Areas outside of the High Potential and Primary Dispersal Zones; sites where last known *B. affinis* record is from before 2000; and unoccupied sites (defined below) with at least 3 years of negative surveys with sufficient effort³ since the last known record. For recommendations regarding protocols and scientific recovery permits in Unoccupied Zones, see Table 1.
- **Unoccupied Sites** are where the species has been previously documented since 2000, but where there have been at least 3 years of negative surveys since the last known record (*i.e.*, using protocol 2 or equivalent effort). Sites that only have records older than 2000 are also assumed to be unoccupied unless new information (*e.g.*, recent surveys) suggests otherwise.

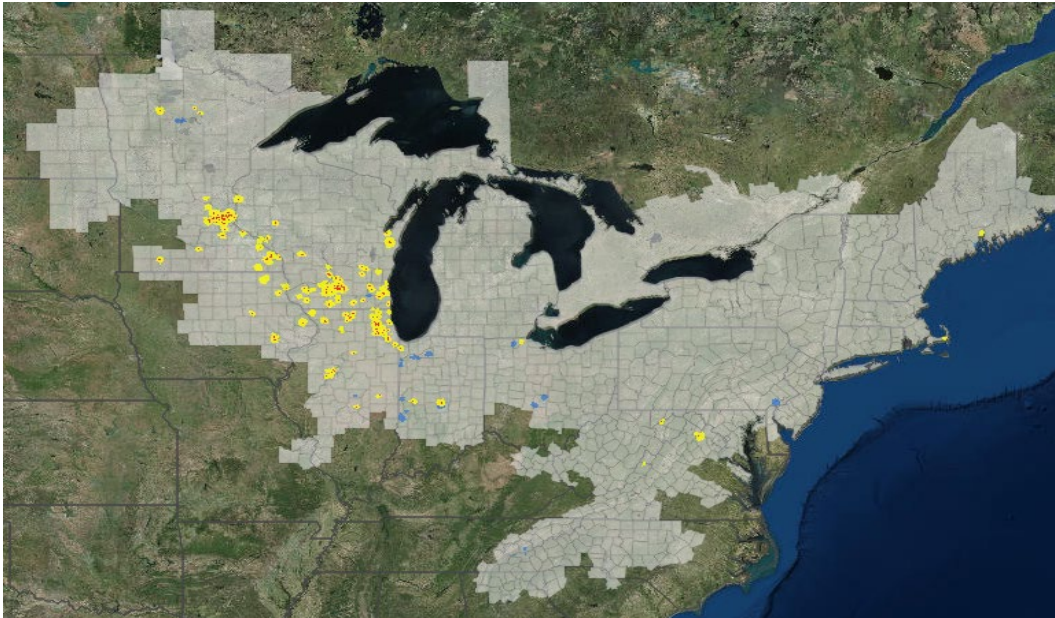


Figure 1. “High Potential Zones” (red dots), Uncertain Zones (blue), and Primary Dispersal Zones (yellow) (together, the Uncertain and Primary Dispersal Zones comprise the “Low Potential Zones”) for *B. affinis*, based on a habitat connectivity model and on species survey data compiled through the previous field season. The gray shaded area is the historical range of *B. affinis*, and is called the “Unoccupied Zone”. This map is updated regularly, so we recommend that surveyors visit the FWS RPBB website for the most current information. Description of the habitat model, an interactive map, and downloadable shapefiles are available on the RPBB website (<https://www.fws.gov/midwest/endangered/insects/rpbb/rpbbmap.html>).

³ Sufficient effort would consist of four approximately equally spaced sampling periods during the sampling season (early June to mid-August); one-person hour of search time per three acres of suitable high quality habitat (defined below) using non-lethal netting techniques. This document provides further details on methods, techniques, and best practices and is subject to continual improvement and modification.

Recommendations for Surveys and ESA Permits within these Zones

Our recommendations for survey protocols and for obtaining a recovery permits within the High Potential, Low Potential, and Unoccupied Zones is described below (also, see Table 1).

High Potential Zone - With respect to typical foraging distances and potential dispersal movements of *B. affinis*, the high potential zones provide a reasonable basis for describing where the species is likely to be present and *where federal agencies and others should consult with the FWS to evaluate the potential effects of their actions* (Table 1). In addition, researchers surveying for or studying *B. affinis* in these zones are advised to obtain a scientific recovery permit.

Low Potential Zone – Since the late 1990s, marked and precipitous declines have been recorded in spatial extent and in the number of existing populations of *B. affinis*. Initial *recovery efforts* are needed around existing populations to avoid extinction of this species. Due to the low number and small size of most existing populations, looking for additional sites to support recovery may help prevent the extinction of this species. Low potential zones buffer high potential zones and are much less likely to support existing populations. However, we are hopeful that *B. affinis* may be found in some of these areas based on observed dispersal distances of a closely related species (*B. terrestris*, 0.6 to 6.2 mi (1 to 10 km, Kraus et al. 2009, p. 249; Lepais et al. 2010, pp. 826-827)). Therefore, since there is some chance (though low) that a surveyor looking for *B. affinis* may find one, we recommend that surveyors in low potential zones obtain a scientific recovery permit (Table 1).

Unoccupied Zones – We believe the likelihood of finding a *B. affinis* in these areas is so low, that we do not recommend scientific recovery permits in these areas (Table 1). However, if an insect researcher is surveying for bees in these areas and is concerned that they may accidentally collect a *B. affinis* and would like to obtain a recovery permit, then they may apply for such a permit and would be authorized to use lethal survey methods, until such time, if ever, that they happen to collect a *B. affinis*. At that time, the permit would specify that they switch to non-lethal methods within the occupied area.

Zone	Purpose	Recommended Protocol	Is a Scientific Recovery Permit Recommended?	Effort (per visit)	Effort (per season)	Duration	Transects	Count or Estimate of each individual bee?	RPBB Habitat Assessment?	Notes
High Potential Zone	"Presence - Absence" Survey for Section 7/10 Consultation/HCP	Project Review	Yes	1 person-hr per 3 acres of best habitat	4 equally spaced sampling periods from mid-June to mid-August	1 flight season, at minimum	No	Estimates or Counts	Not required, but recommended	
High Potential Zone	Bumble bee community and <i>B. affinis</i> population monitoring	Recovery Monitoring	Yes	1 person-hr per 3 acres of best habitat	4 equally spaced sampling periods from mid-June to mid-August	3+ years	Optional	Counts	Highly Recommended	
High Potential Zone	Document bumble bee presence without handling <i>B. affinis</i>	Photo Only	No	At your discretion	At your discretion	At your discretion	No	Estimates or Counts	Not required	A photo only can only verify presence, so the survey may not provide sufficient effort necessary for project reviews.
Low Potential Zone	Bumble bee community and population monitoring	Recovery Monitoring	Yes	1 person-hr per 3 acres of best habitat	4 equally spaced sampling periods from mid-June to mid-August	3+ years	Optional	Counts	Highly Recommended	
Low Potential Zone	Document bumble bee presence without handling <i>B. affinis</i>	Photo Only	No	At your discretion	At your discretion	At your discretion	No	Estimates or Counts	Not required	
Unoccupied Zones	Find new locations of <i>B. affinis</i>	Rapid	No	1 person-hr per 3 acres of best habitat	One time visit	1 flight season, at minimum	No	Estimates or Counts	Not required	If <i>B. affinis</i> is observed, stop survey and notify USFWS.
Unoccupied Zones	Document bumble bee presence without handling <i>B. affinis</i>	Photo Only	No	1 person-hr per 3 acres of best habitat	At your discretion	At your discretion	No	Estimates or Counts	Not required	If <i>B. affinis</i> is observed, stop survey and notify USFWS.
Unoccupied Zones	Other bee surveys	See Protocol Suggestions for Unoccupied Zones	No	At your discretion	At your discretion	At your discretion	No	At your discretion	Not required	If <i>B. affinis</i> is observed, stop survey and notify USFWS.

Table 1 . Quick guide to protocol recommendations based on the location and purpose of the survey, including recommendations whether or not to obtain a scientific recovery permit. The primary differences between the protocols are the amount of effort that is recommended and whether or not you anticipate handling *B. affinis*. For each protocol, the recommended amount of effort per field visit, effort per active season (year), survey duration, and habitat assessment is given. We recommend that surveyors conduct detailed habitat assessments (available online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/pdf/HabitatAssessmentFormGuideByXercesForRPBB.pdf>) at sites with a positive identification of *B. affinis*.

Guidelines and Methods

General Guidelines and Best Practices for Surveys

We recommend following the guidelines below.

- **Adhere to the Endangered Species Act and determine if you need a survey permit.**
Details on how to apply for a permit are provided online at -**Obtaining a Recovery Permit under the ESA and FAQs** (<https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html>).
FWS only has regulatory authority over listed species.
- **Obtain land-owner permission.** Written permission is recommended.
- **Conduct surveys during suitable survey conditions** (below).
 - **Time of Year** - Surveys (if handling *B. affinis*) must be conducted between early-June and mid-August, for the highest detection probability and to reduce potential impacts to *B. affinis* queens.
 - **Weather** - Surveys should take place when temperatures are above 60°F (15.5°C) and not during wet conditions (*e.g.*, foggy, raining, or drizzling). Wait at least 1 hour after rain subsides before conducting a survey. Sunny days with low wind speeds (less than 8 mph) are optimal. Partially cloudy days or overcast conditions are permissible if you can still see your shadow.
 - **Time of Day** - Surveys should be conducted at least 2 hours after sunrise and 3 hours before sunset.

Netting Technique. Collect bumble bees directly from flowers. Videos on netting techniques are available here: <https://www.youtube.com/watch?v=SwYbv5bySPQ>. If possible, capture one bee at a time or immediately separate bees into individual containers.

- **Handling.** Do not hold a bee in a container for longer than 15 minutes, unless you place it in a cooler with ice to later photograph the bee. Hold only one bee per container. Do not hold bees in a cooler with ice for more than 2 hours and do not place bees directly onto ice. If the air temperatures are above 90°F (32°C), do not hold for longer than 5 minutes. Bumble bees can easily overheat, so do not keep vials/bags in direct sunlight. Keep the bees in the shade, if possible. Be careful not to get stung!
- **Release.** Release bees back on or near flowers on which they were found. If you held bees in a cooler with ice, first transfer bees to a warm container after being in cooler (as the original container will fog up when it hits the warm humid air). Place the open vials (or bags) in a shaded area to allow the bees time to warm up and fly away. Release *B. affinis* within 15 minutes of capture, if possible.

- **Reporting.** Report sightings of *B. affinis* as required on your permit(s). Any collections or sightings of *Bombus bohemicus* (formerly *B. ashtoni*) should also be reported as this is a nest parasite of rusty patched bumble bee (and *B. terricola*) and thus could potentially indicate the presence of *B. affinis* in the area. Although not required, FWS is also requesting that surveyors report *B. terricola* observations and flower use.
- **Salvage.** *B. affinis* that are found dead or killed accidentally (during authorized surveys) may be salvaged. Prior to collecting specimen(s), photograph the specimen to document the condition. Notify the USFWS within 48 hours. Preserve the specimen(s) using standard museum practices⁴ including proper identification and data [include date, complete scientific and common names, and geographic location (township, range, section, and UTM) where salvaged], or place in a freezer if unsure how to preserve. All specimens of *B. affinis* collected under your federal permit are the property of the United States Government and should clearly be identified as such. All dead specimens should be sent to a public scientific or educational facility or museum in the state the individuals were collected along with a copy of the permit(s) under which they were collected.
- **Equipment basics** – We suggest bringing the following equipment:
 - **Nets.** Use cloth aerial hand nets. Do not use sweep, beater, or wire nets. Netting should be fairly transparent. In rare cases, some individuals may be permitted to directly collect *B. affinis* into vials (see **Obtaining a Recovery Permit under the ESA and FAQs** available online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html>).
 - **Bags and/or clear vials.** If using vials, make sure vials allow for clear photographing of specimens inside (test before you go into the field). If using plastic bags, bring extra bags in case moisture builds up or bags get wet. There will be sufficient air in any of these containers while taking photographs.
 - Binoculars - butterfly binoculars are recommended for visual surveys
 - **Camera** (see **Appendix A - Standardized Bee Photography** for more details)
 - **Cooler with ice, if applicable**
 - **GPS unit**
 - **Data sheets** (digital or paper, for both bee surveys and habitat assessments)
 - **Pencils**
 - **Timer or stopwatch**
 - **Hand lens or loupe**
 - **Long measuring tape (100+ft)**
 - **Flags or stakes to mark transects**
 - **Field guides**
 - Federal, State, and Local **Permits, if applicable**

⁴ You may find tutorials and protocols on the World Wide Web, for example, see methods on the University of Minnesota webpage <http://www.extension.umn.edu/youth/mn4-H/projects/environment/entomology/collecting-and-preserving-insects/>

Methods for Surveys within the High and Low Potential Zones

The following methods are common to all of the recommended protocols in this section (Table 1). Methods specific to each protocol are provided under each protocol description, below.

Site Selection

- Site selection will depend on the objectives and purpose of your study. Examine recent aerial photography using Google Earth or other tools to identify potential habitat for bees (see **Appendix B - Habitat for *B. affinis***). Examine and consider vegetation surveys or floral lists, if available for the location.

Define the Survey Area

- Determine the area that you would like to survey. The size of the survey area is at your discretion, but sufficient effort should be applied to the amount of area surveyed (see **Survey Effort**, defined for each protocol, below).
- On the field sheet, roughly estimate the size of the surveyed area and/or sketch site. Delineate the survey area using a GPS program such as ArcGIS to draw polygons around the area sampled.
- Use a portable GPS device (or map) to record the coordinates (decimal degrees is preferred) from, at minimum, the four corners of the surveyed area and at the approximate center of the surveyed area (latitude of eastern and western boundaries and longitude of northern and southern boundaries).

In Field Habitat Assessments

- If possible, prior to the survey date (if time permits), field-truth the selected area. Check the area for floral resources.
- On the day of the survey, take representative photographs, showing areas of suitable (high quality) and unsuitable (poor) habitat.
- At minimum, collect the basic habitat information on the **Survey Protocol Data Sheets** (available online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html>) to quantify the bees observed and survey conditions, which can affect the species observed. The sheets also ask for basic information about the available floral resources and major stressors at the site.
- You may conduct more detailed habitat assessments at your discretion – we recommend using the Xerces Rusty Patched Bumble Bee Habitat Assessment Form and Guide (<https://www.fws.gov/midwest/Endangered/insects/rpbb/pdf/HabitatAssessmentFormGuideByXercesForRPBB.pdf>). This Assessment can help conservation planners and landowners prioritize conservation actions and quantify habitat or land management improvements for *B. affinis* on a single site. It also helps identify specific actions for habitat improvement and management practices to help protect *B. affinis* from potential threats. We recommend that surveyors conduct detailed habitat assessments, particularly at sites with a positive identification of *B. affinis*.

Record Floral Use

- Note the flower (species of plant) being used by any *B. affinis* found, if applicable. If you are unsure of the plant species or need help with plant identification, photograph the flower and diagnostic parts (*e.g.*, stem and leaves) to aid with later identification.

Photographs

- Take several photographs of each *B. affinis* (and other species of interest in your area, *e.g.*, *B. terricola* and *B. pensylvanicus*). Photographs are required to verify *B. affinis* observations.
- Representative photographs of each bumble bee species in each sampling location is highly preferred.
- Photograph the bee from the top (dorsal view) showing the entire bee, including the top of thorax and abdomen. Also photograph the face from the front and top, and side view of thorax and abdomen (**see Appendix A - Standardized Bee Photography**). Short videos of *B. affinis* are also recommended.
- If using vials, make sure vials provide clear pictures (test before you go into the field).
- Record GPS location with each associated observation.

Survey Methods

- Start your timer when you begin surveying.
- **Catch bumble bees according to protocol specifications**, preferably one at a time. Move bee from aerial net to clear vial, baggie, or plastic bag.
- Pause your timer while you are not actively searching for bees (*e.g.*, while you are putting bees in your cooler, walking between habitat patches, or taking photographs).
- Depending on the specifics of your survey, you may choose to use blue chalk dust, paint pens, or nail polish to temporarily mark bees, other than *B. affinis*, to avoid recapture of individual bees (*e.g.*, if you process bees right away). Do not mark bees on the wings. In some cases, marking *B. affinis* may be permitted for individuals highly experienced in marking bumble bees - contact your state's FWS Ecological Services Field Office, if this is something you are considering.
- In some cases, visual surveys (*e.g.*, using butterfly binoculars) to count or estimate species other than *B. affinis* (and *B. terricola*) may be the preferred method for individuals highly experienced in identifying bumble bees. Photographic documentation is a necessary secondary component to visual surveys (*e.g.*, if *B. affinis* is observed).

Identification and Verification

- **See Appendix C – Bee Identification.**
- **See Appendix D - How to Verify Records.**

Release

- Release all *Bombus* at site of capture, on or near flowers (see *Release* in **General Guidelines and Best Practices for Surveys**, above).
- Process and release *B. affinis* within 15 minutes of capture, if possible.

- You may choose to release other species of *Bombus* at the end of the survey to avoid double counting individual bees.

Reporting

- **Report** all surveys (positive and negative) as required on state and federal permits.
 - We recommend submitting the **Survey Protocol Data Sheets** (available online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html>) for annual permit reports. Using the data sheets will help standardize data collection and increase efficiency in reporting.
- **Record** survey and habitat information on **Survey Protocol Data Sheets** (available online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html>).
- **Contact the FWS Field Office** near you (<http://www.fws.gov/offices/>) if you find and verify a *B. affinis* observation, particularly if it is observed in a new location.

Survey Protocols

Photograph Only Surveys

*Photo-only surveys are recommended for people who will not be handling *B.affinis*. You do not need a permit if you do not handle *B. affinis*. Surveys to find new populations should place emphasis on maximizing the possibility of finding additional *B. affinis* populations through surveying many areas, rather than repeated sampling in one area (Table 1). Although photographic surveys can be conducted anywhere, we do not recommend photo only surveys for project reviews.*

- We recommend using the photograph techniques described in **Appendix A - Standard Bee Photography**.
- We recommend that you submit photos to Bumble Bee Watch (www.bumblebeewatch.org), BeeSpotter (<https://beespotter.org>), or a similar website that employs bumble bee experts to verify the identifications. Qualified scientific experts may also be used to verify photographic records.
- Only good photographs that show key characteristics will be verified.
- Notify FWS Field Office near you (<http://www.fws.gov/offices/>) as soon as possible if you observe *B. affinis*, particularly if you observe it in a new location.
- Try to count and photograph all *B. affinis*, at minimum estimate the number of *B. affinis* observed. Note the flower that *B. affinis* is using.
- If possible, estimate and note the numbers observed of each species as well as the flower(s) the bee was using.
- Photographic documentation of *B.affinis* is required to verify the record.

Rapid Survey Protocol – Surveying Areas to find New Populations

*Surveys to find new populations should place emphasis on maximizing the possibility of finding additional *B. affinis* populations through surveying many areas, rather than repeated sampling in one area (Table 1). Rapid surveys are recommended for quick searches for *B. affinis* within the **Unoccupied Zones**. You do not need a permit in the Unoccupied Zones. Depending on the purpose of your study, you may also choose to use other protocols, such as those described below.*

Site Selection – If you have discretion on where you will conduct surveys, we recommend prioritizing your survey locations near recent records of the species and in areas with high quality habitat in your geographic area of interest in the **Unoccupied Zones**. Contact the USFWS Field Office (<http://www.fws.gov/offices/>) to help find priority areas within in your area of interest or use our guidance to help identify **priority survey areas** (online at <https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html>).

Distance Between Sites – If you would like to survey multiple sites, spacing of sites will depend on the size of the survey area you are covering and the available time. In general, we suggest that surveyed sites be spaced approximately 3 to 5 km (2 to 3 mi) apart to maximize detection of regional populations of *B. affinis*.

Survey Effort – Survey for at least **one (1) person-hour per three acres of highest quality habitat** in your survey area or continue to sample until at least 150 bumble bees are sighted, whichever comes first⁵. Spend minimal time in lesser quality habitat. Scan the areas for bumble bee activity and move towards those areas. If bumble bees are not obvious, then travel from flower patch to flower patch looking for active bumble bees. Do not spend more than a minute at any one patch if you don't see any bumble bee activity. Because the purpose of the **Rapid Protocol** is to find new locations for the species, preferentially capture bees that match descriptions of *B. affinis*.

Survey Technique and Methods – *Use non-lethal netting techniques.*

- Because the purpose of the **Rapid Protocol** is to find new locations for the species, **preferentially capture bees that match descriptions of *B. affinis***. Catch bumble bees of interest, preferably one at a time.
- You may use visual surveys to count or estimate numbers of each species besides *B. affinis* (and *B. terricola*), however, this method is only advised for individuals highly experienced in identifying bumble bees in the field and is recommended only for commonly observed species. Indicate degree of confidence in identifications on the data sheets. Photographic documentation of *B. affinis* is required to verify the record.
- As you are surveying, make a mental note of other *Bombus* species observed, if not captured for photographs. The data sheet will provide space to approximate the numbers of each species observed. Note how these were counted (i.e. actual counts or estimates) on the data sheets, to ensure that numbers are not misinterpreted.

⁵ See Appendix E - Further Information Regarding Sampling Effort.

Project Review Survey Protocol

*Surveys to confirm extant or uncertain site locations should have a standard level of effort at any given visit, plus repeated sampling during at least one year of the active flight season (Table 1). Because the purpose of these surveys is to detect B. affinis, sampling may be biased towards finding that species, however a full Bombus species list is also requested. Surveys should be conducted within a year of project initiation, however locations can be surveyed for multiple years (e.g., if you want to be reasonably certain that the species no longer exists in the area, see definitions of an unoccupied zones and unoccupied sites, above). More information is given in **Appendix F –About Surveys for Proposed or Ongoing Actions that May Impact B. affinis**.*

Site Selection – Select sampling locations within the High Potential Zone or Low Potential Zone.

Survey Effort - Conduct surveys during four evenly (approximately) spaced sampling periods during the sampling season (as defined in the **General Guidelines and Best Practices** section, above). For each sampling event, survey each suitable habitat patch for a **one (1) person-hour per three acres of the highest quality habitat** in your survey area or continue to sample until at least 150 bumble bees are sighted, whichever comes first⁶. Because the purpose of Project Review Protocol is to find new locations for the species, preferentially capture bees that match descriptions of *B. affinis* and estimate the number of other *Bombus* species. Surveys should be conducted within a year before the project initiation for negative survey results to remain valid for the duration of the project unless new information (e.g., new positive surveys) suggests that the species is likely to be present in the action area. Although not required for project review, repeat sampling events in the same areas (if still suitable) for at least 3 consecutive years are advisable if you are trying to be reasonably certain that the species no longer occupies the area (see **Unoccupied** site in **Definitions** section, above).

Survey Technique and Methods – *Use non-lethal netting techniques*

- **Preferentially capture bees that match descriptions of *B. affinis*** and other species that are of particular interest to you. Catch bumble bees of interest, preferably one at a time.
- As you are surveying, note of other *Bombus* species observed, even if not captured for photographs. The data sheets provide space to approximate the numbers of each species observed. Note how each species was counted/approximated on the data sheets, to ensure that numbers are not misinterpreted.
- You may also choose to capture other species to help you count and photographs them.
- You may use visual surveys to count or estimate numbers of each species besides *B. affinis* (and *B. terricola*), however, this method is only advised for individuals highly experienced in identifying bumble bees in the field and is recommended only for commonly observed species. Indicate degree of confidence in identifications on the data sheets. Photographic documentation of *B.affinis* is required to verify the record.

⁶ See Appendix E - Further Information Regarding Sampling Effort.

Recovery Monitoring (*Bombus* Community) Survey Protocol

Long-term monitoring should be conducted in extant sites or any site where you are interested in population trends, species richness, and relative abundance of all Bombus species over time (Bombus community data)(Table 1). Surveys should have a standard level of effort in order to detect changes in relative abundances over time. Surveys should be conducted multiple times per year for multiple years. Here, we give three options - one using transects, one without transects, and distance sampling with transects.

Background – Recovery monitoring can be conducted to detect trends in known populations at **extant sites or at other sites at your discretion**. The quantity of bumble bees changes throughout the warm months as worker populations increase or decrease. For example, bees are subject to many environmental and health factors that impact the number of workers produced by each bumble bee colony. Additionally, over time, bumble bee habitat suitability changes as floral landscapes change composition. Because suitable habitat may change locations from one year to the next and bumble bee numbers fluctuate throughout the season, quantifying populations can be difficult and those variabilities should be accounted for in your monitoring effort.

Define the Fixed Survey Area - Determine and define your long-term survey area. This survey area should remain fixed across years. Focus survey effort in areas with the highest quality habitat.

Define and Map Suitable Habitat – High quality habitat should be delineated on a map or drawing each year (see **Appendix B - Habitat for *B. affinis***).

Survey Effort - Conduct surveys during four evenly (approximately) spaced sampling periods during the sampling season (as defined in the **General Guidelines and Best Practices** section). Survey all or a random subset of the highest quality habitat in the survey area. If the survey area is greater than 50 ac (20 ha), divide the survey area and survey separately in increments of 50 ac (20 ha). We recommend that surveyors use one of the three options below:

- **Option 1 - Surveying with Transects:** We recommend surveying transects (meandering transects are okay) that can be repeatedly visited over the course of the survey season and/or multiple years.
 - Maintaining the exact location of each transect is not as important as recording the transect length and width because these measurements will give us an estimate of the area sampled.
 - Multiple transects at each site is recommended to adequately cover the highest quality habitat at the site; transect spacing will depend on the habitat. You may preferentially place transects through good floral patches.
 - Use a GPS unit to track transects (it will give you transect length), or flag the transect and measure the transect length using a measuring tape.
 - For each 100m transect length (we recommend a minimum length of 300m), survey for a minimum of 15 minutes, use more time if needed; capture bees within 1 meter of one side of the transect line.

- Record the amount of time spent sampling each transect each visit.
 - Repeat sampling events in the same areas (if still suitable) for at least 3 consecutive years.
- **Option 2 - Surveys Without Transects:** Alternatively, if you are not using transects, for each sampling event, survey for a minimum of **one (1) person-hour per three acres of the highest quality habitat** in the survey area or continue to sample until at least 150 bumble bees are captured, whichever comes first⁷. Estimate the area of the high quality habitat sampled. Repeat sampling events in the same areas (if still suitable) for at least 3 consecutive years.
- **Option 3 – Distance Sampling with Transects:** We recommend surveying transects that can be repeatedly visited over the course of the survey season and/or multiple years. Hierarchical Distance Sampling (HDS) has been used to estimate bumble bee densities (e.g., McNeil et al. 2018) and allows for spatial variability in abundance and detection across sites to be explained as a function of covariates (Kéry & Royle [2015](#)).
 - Maintaining the exact location of each transect is not as important as recording the transect length and width because these measurements will give us an estimate of the area sampled.
 - Multiple transects at each site is recommended to adequately cover the highest quality habitat at the site; transect spacing will depend on the habitat. You may preferentially place transects through good floral patches.
 - Use a GPS unit to track transects (it will give you transect length), or flag the transect and measure the transect length using a measuring tape.
 - For each 100m transect length (we recommend a minimum length of 300m), survey for a minimum of 15 minutes, use more time if needed; capture/count bees within 3m of one side of the transect line.
 - Record distances as the perpendicular distance (to the closest 0.25m) from the transect to each bee and note the distance at which the bee was *first* detected. Avoid double-counting individuals.
 - Record the amount of time spent sampling each transect each visit.
 - Repeat sampling events in the same areas (if still suitable) for at least 3 consecutive years.

Survey Technique and Methods for both options– Use non-lethal netting techniques

- **Catch all bumble bees observed**, preferably one at a time.
- **Carefully count numbers of other *Bombus* species** captured. This information will be used to calculate relative abundances.
- You may use visual surveys to count or estimate numbers of each species besides *B. affinis* (and *B. terricola*), however, this method is only advised for individuals highly experienced in identifying bumble bees in the field and is recommended only for commonly observed

⁷ See Appendix E - Further Information Regarding Sampling Effort.

species. Indicate degree of confidence in identifications on the data sheets. Photographic documentation of *B.affinis* is required to verify the record.

Habitat assessment - Within each defined survey area each year, we ask that surveyors conduct a detailed habitat assessment provided online at (<https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html>). You may also use the “detailed habitat assessment” tab on the data sheets available online. Map suitable habitat within your survey area illustrating (1) area of high quality habitat and (2) amount of low quality habitat.

Protocol Suggestions for Unoccupied Zones

*Here, we recommend established protocols for bumble bee surveys and inventories in areas within the **Unoccupied Zones**, within the historical county range, or beyond the boundaries where the species was thought to occur. Your choice of technique is based on the objectives of the study and is not limited to those listed here. Survey Protocols discussed above may also be conducted outside of the Unoccupied Zones, if you desire.*

Some Considerations. Research on bees or pollinators in general often uses lethal survey techniques including hand netting, bowl traps, malaise traps, and window traps. Such techniques, while lethal, are thought not to significantly impact most (*e.g.*, relatively healthy) bee populations following the survey year (*e.g.*, Gezon et al. 2015, pp. 4-6). Passive traps (such as those discussed below) should still be used with caution and common sense. **Any situations where queens may be harmed should be avoided.**

Site Selection – Sites should be within the **Unoccupied Zones** (see Survey Protocol map, <https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>), updated by February 28th of each year).

Time of the Year - Sampling may occur any time during the active season of bees (primarily March-October).

Recommended Survey Protocols

See the USFWS National Protocol Framework for the Inventory and Monitoring of Bees (<https://ecos.fws.gov/ServCat/DownloadFile/47682?Reference=47400>) or for more details on some of the protocols listed below.

- **Netting at flowers with kill jars**- Bumble bees can be netted on flowers using timed intervals, transects or a combination. Potassium cyanide or soapy water are preferred as DNA is not degraded but ethyl acetate and calcium carbonate may also be used. See the Bumble Bee Megatransect Project (<https://www.handsontheland.org/environmental->

[monitoring/bumble-bee-megatransect.html](https://www.fws.gov/ecos/ServCat/DownloadFile/47682?Reference=47400)) or, more generally, roadside surveys (<http://www.slideshare.net/sdroege/bumblebee-roadside-surveys-a-pilot-survey-and-recommendations>),

- **Bowl/Pan/Cup traps** - These are traps that are usually colored blue, yellow, and white and set out on the ground or slightly elevated. Bees are attracted to the color and drown either in soapy water or diluted propylene glycol. Bumble bees are captured relatively uncommonly in most bowl and pan traps. However, cup traps, deployed continuously, often collect reasonable number of bumble bees and are a good choice for long-term surveys. Their use is outlined in the National Protocol Framework for the Inventory and Monitoring of Bees (<https://ecos.fws.gov/ServCat/DownloadFile/47682?Reference=47400>).
- **Malaise traps** - Use of tent-like traps made of mesh fabric that act as flight intercept traps with insects funneled into a collecting head filled with alcohol, glycol, no-pest strips, or cyanide. Costs for individual traps are high thus they are not commonly deployed despite capturing substantial number of bumble bees. Catch is highly dependent on location and habitat type.
- **Quick assessment** of bees at planted sites (*i.e.*, Ward et al. 2014) can be found at (http://www.xerces.org/wp-content/uploads/2014/09/StreamlinedBeeMonitoring_web.pdf).
- **Other passive survey techniques** occasionally catch or are used to collect bees. These include pheromones, window pane traps, sticky traps, pitfall traps, etc. These usually catch only very small numbers of bees or bees other than *Bombus* and the catch is often accidental.

Other Techniques (not recommended)

- **Blue Vane Traps** - Plastic bucket-like traps with fluorescent blue vanes. The bucket contains no-pest strips or propylene glycol as a kill agent. Blue vane traps can, at times, collect large numbers of bees when floral resources are limiting and should be used with caution during times when queens are out. Blue vane traps should be particularly used with caution throughout the historic range as they can catch bumble bees and *Melissodes* (also *Eucera* and *Peponapis*, *R. Jean pers.comm. 2018*) in large numbers. However, blue vane traps can also be used to capture bees alive, if they are monitored throughout the day to avoid stress from heat, etc. At least two *B. affinis* records were documented to be caught in blue vane traps (USFWS unpublished geodatabase) and bumble mortality is documented from blue vane traps (*e.g.*, Gibbs et al. 2017).

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Appendix A – Standardized Bee Photography

- Type of camera - Point and shoot digital cameras, DLSR and phones with cameras (≥ 8 megapixels) will be the best options for use after aerial netting and moving the bee into a clear vial or clear baggie (zip net) to take photos.
- To properly identify a bumble bee it is best to take photos that clearly show the entire top side of the abdomen, the side of the thorax/abdomen and the face/head. Take several photos of each specimen to show these various characteristics.
- Hair color patterns vary with lighting. Examine photos to ensure that coloration is clear and that shadows and the underlying integument are not creating deceptive color patterns.
- If you are not handling bees, photograph them as they forage, taking a series of photos of each individual to document the bee's characteristics clearly, as described above. Take a "spacer" photo between the series, so you can distinguish between individual bees when you archive photos and report findings.
- If you are handling bees, vials with a flat side may provide higher quality photos. Placing the bee in a cooler will slow them down making them easier to photograph. You need a scientific recovery permit to handle *B. affinis* within High and Low Potential zones (see Table 1).
- Do not hold a bee in a container for longer than a few minutes, unless you place it in a cooler with ice. Bumble bees can easily overheat, so do not keep vials/bags in direct sunlight. Do not keep bees in a cooler longer than two hours, and not directly on the ice.
- If using a cooler with ice, bees may take a few minutes to warm up before flying away. Place vials or bags in a shaded area to give the bees time to fly away.
- Archive photographs for long-term storage.
- Link each photograph to the corresponding specimen. Use standardized naming conventions that provide the following information:
 - Permit number
 - Site location code
 - Specimen number
 - Photograph number
 - Date
 - For example, label PERMITNUMBER_SITECODE_SPECIMEN#_PHOTO#_DATE (e.g., TE5555-7_STPAUL28_Specimen12_photo2_12July2017)

Appendix B - Habitat for *B. affinis*

Bombus affinis has been observed and collected in a variety of habitats, including prairies, oak savanna, woodlands, marshes, parks, and residential areas (Colla and Packer 2008, p. 1381; Colla and Dumesh 2010, p. 46; USFWS rusty patched bumble bee unpublished geodatabase 2016). *B. affinis* needs areas that contain sufficient food (nectar and pollen from diverse and abundant flowers), nesting sites that are predominantly free from ground-disturbing activities and near floral resources, and overwintering sites for hibernating queens (Goulson et al. 2015, p. 2; Potts et al. 2010, p. 349). It is a generalist forager for pollen and nectar like other bumble bees (Xerces 2013, pp. 27–28), but relies on diverse and abundant flowering plant species (Goulson et al. 2015, p. 2; Potts et al. 2010, p. 349). Due to the early emergence of *B. affinis* (roughly, mid-March through April), woodlands that support early blooming spring ephemerals are likely important habitats (Colla and Dumesh 2010, p. 45-46), especially when they are near open areas that are also used for summer foraging. *B. affinis* nests are typically in abandoned rodent nests or other similar cavities, one to four feet below ground (Plath 1922, pp. 190-191; Macfarlane et al. 1994, p. 4). *B. affinis* nests have also been occasionally observed above ground (Plath 1922, p. 190). Little is known about the overwintering habitats of *B. affinis* foundress queens, but other species of *Bombus* typically form a chamber in soft soil, a few centimeters deep (often in forests or in forest edges) and sometimes use compost or mole hills to overwinter (Goulson 2010, p. 11). Overwintering (roughly, mid-October through mid-March) sites may typically be in loose, uncompacted and often sandy, moss-covered soils on northwest exposures (E. Evans, University of Minnesota, pers. comm. 2017).

While *B. affinis* will use early spring floral blooms near the edge of forests and woodlands, ultimately they are dependent on open habitats to complete their life cycle. Consequently, potentially suitable habitat can be defined as any open, vegetated habitat within your survey region, such as prairies, roadsides, and meadows (Figures B.1, B. 3 and B.4). However, open habitats vary in quality for bumble bees. Open habitats with high floral diversity and abundance should be considered “high quality” habitat (Figures B.1, B. 3 and B.4). Areas dominated by grasses or sedges with few flowering plants or a low diversity of flowering plants should be considered low quality.

Habitat can be characterized in part by percent of vegetative cover that is forbs, flowering shrubs, or pollinator-friendly trees; especially areas that bloom all season long. Estimates of the number of species of forbs, flowering shrubs, or pollinator-friendly trees in bloom during each survey can help define “high quality” habitat and can help surveyors focus their effort (see **Survey Protocol Data Sheets** available online at

<https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html>).

Surveyors should spend less time in poor and/or questionable habitat. Questionable habitat includes areas that are not clearly poor habitat, and should be checked for 5-10 minutes for bumble bee activity (Figures B2.a and B2.b) before moving on to better habitat or deciding not to survey an area. Poor habitat can be defined as areas without an abundance of floral resources, areas with

compacted soils, sod-forming grasses, or large monoculture agricultural fields (although some flowering agricultural crops, like alfalfa, may provide foraging habitat). Some examples of poor habitat include open water, regularly maintained turfgrass monocultures, and pavement. Areas that meet the following descriptions are not suitable for the rusty patched bumble bee for nesting, overwintering, or foraging.

- permanently flooded areas/open water;
- paved areas;
- mowed turf lawns without clover;
- areas planted to annual row crops, such as corn and soybeans which do not provide mass flowering resources for bees;
- forest where invasive shrubs are dominant and spring ephemeral flowers are absent; and,
- areas mowed too frequently to allow development of diverse wildflower resources (*e.g.*, road shoulders).

In addition to the above, wetlands, where standing water may be absent but near the ground surface, are unsuitable for nesting or overwintering. Some wetland areas, however, could provide function as important foraging habitat.

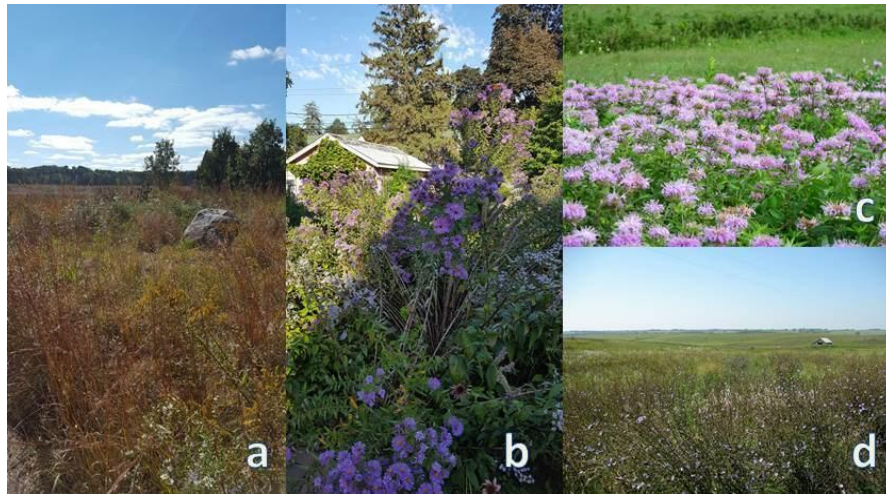
Special note on urban areas: Some of the last refuges for *B. affinis* appear⁸ to be in large urban areas, such as Minneapolis/St. Paul, Madison, Milwaukee and Chicago. From a landscape perspective, these cities have a network of natural areas that include parks, greenways, public gardens and other public or undeveloped lands. Interspersed among these natural areas are residential areas - - yards, gardens and boulevards that provide additional sources of flowering plants and nesting/overwintering habitat – and habitat for dispersal (Fig. B2.b). Areas considered high quality habitat in urban areas have the same characteristics as high quality habitat outside of urban areas. They are generally open areas with an abundance and diversity of plants that flower from mid-March through mid-October; that have undisturbed areas without landscaping mulch or landscape fabric; and that are managed with minimal use of pesticides; particularly insecticides and fungicides.

In the urban landscape, high quality habitat is most likely in or near natural areas that support open, or mostly open, habitats such as prairie, savannas, grasslands, or grassland/shrub mix (Fig. B1.a). Small woodlots and the edges of larger tracts of forested lands also provide high quality habitat if located adjacent to areas with abundant flowering plants or have interspersed meadows. These woodlots or wood edges may provide important early spring habitat if they support spring ephemerals or early spring blooming trees and shrubs. Natural areas within urban areas may be in blocks (small or large) or may be linear. In general, the larger the block of contiguous habitat, the higher the quality the habitat is. The value of any of these tracts is higher if surrounding areas also provide flowering plants and some undisturbed areas, such as residential

⁸ Some urban areas have been surveyed more than other areas, indicating that there may be a sampling bias. On the other hand, many additional (*e.g.*, rural) areas have been intensively surveyed and have resulted in no/few observations of *B. affinis*.

areas with gardens. The habitat quality of small or linear tracts may be negated if surrounding areas are dominated by roads and buildings with little to no natural areas or gardens.

Roadsides and rights-of-way may provide open habitats (Fig. B1.c), but their value is dictated by the type of vegetation (quality is poor if dominated by grasses) and frequency of mowing or other management actions. Rights-of-way may provide better habitat than roadsides because they are mowed or otherwise maintained less frequently. Rights-of-way may also provide corridors of dispersal, allowing for genetic exchange among sites.



Figures B1 a - d. Examples of high quality habitat and/or connectivity habitat for *B. affinis*; (a) native prairie habitat within an urban landscape, (b) large patch of diverse floral resources within an urban landscape for connectivity, (c) large patch of wild bergamot along a roadside, and (d) open meadow with an abundance of floral resources. Photographs were taken by T. Smith, USFWS (a,b) and Rob Jean, ESI (c,d).



Figures B2 a - b. Examples of questionable habitat for *B. affinis*; (a) area that appears dominated by sod grass (b) predominantly grassy area with little or no floral resources. These areas should be checked for approximately 5 minutes for bumble bee activity during the flight season. If no activity is observed, we would not recommend a survey in these areas. Photographs were taken by Rob Jean, ESI.

Appendix C – Bee Identification

This section lists basic key diagnostics for field identification. These should be documented clearly when using photography survey protocols. For detailed and technical descriptions, see **Bumble Bees of North America: An Identification Guide** by Paul H. Williams, Robbin W. Thorp, Leif L. Richardson and Sheila R. Colla; and Discover Life (<http://www.discoverlife.org/mp/20q>). Other helpful resources include: <http://pollinator.org/PDFs/BumbleBeeGuide2011.pdf>, <http://www.xerces.org/bumble-bee-identification/> and <https://www.flickr.com/photos/usgsbiml/sets/72157664851159091>.

Bombus affinis gynes and queens are entirely yellow on the first two abdominal segments; the rest of the abdominal segments are black (Figure C.1). In workers and males, the first abdominal segment is yellow, and the second has a medial patch of rusty hairs on the anterior portion of the segment, with yellow hairs on the posterior portion (Figure C.1). *B. affinis* can vary from pale tan to a bright rusty brown. The other abdominal segments are black. Occasionally, abdominal segments 3-6 may have reddish hairs or a reddish patch.

B. affinis has a mostly yellow upper thorax with a black spot or band that may extend toward the posterior in a v-shape. The bottom of the thorax is black. Queens and workers have black hairs on the head and at the back of the head. Males have black hairs on the head, with some yellow hairs intermixed at the back of the head. Overall, hairs are moderately long and even.

B. affinis is a short-tongued species, and sometimes is observed nectar-robbing on tubular flowers. It has a short face, with cheek (oculo-malar area) slightly shorter than broad.

B. affinis phenology in each region will determine when queens (or gynes), workers, or males will be observed in flight. For example, in southern Wisconsin, *B. affinis* gynes and queens, distinguished by larger size and other characteristics described above, are in flight in spring (roughly mid March - May) and then again in late summer and fall. Workers can be seen in the field several weeks after nest establishment, throughout the summer, into early fall (late June-September). Males are in flight in late summer and fall (August-September).

More difficult to observe, nesting occurs mid - March, April or May through September, and gynes overwinter from August until mid- March, April or May. If you happen to observed activity of *B. affinis* gynes and queens, take notes regarding behavior, habitat use, etc., as nesting and overwintering are little known. Notify FWS as soon as reasonably possible.

B. affinis can be confused with *B. citrinus*, *B. griseocollis* (Fig. C.3), *B. perplexus*, and *B. vagans*.



Figure C.1. Illustrations of a *B. affinis* queen (left), worker (center), and male (right) by Elaine Evans, University of Minnesota.



Figure C.2. Photographs of *B. affinis* (a) top view, (b) side view and (c) side/top view. Photographs were taken by Dan Mullen (Creative Commons) (a,b) and USGS Bee Monitoring Laboratory (c).

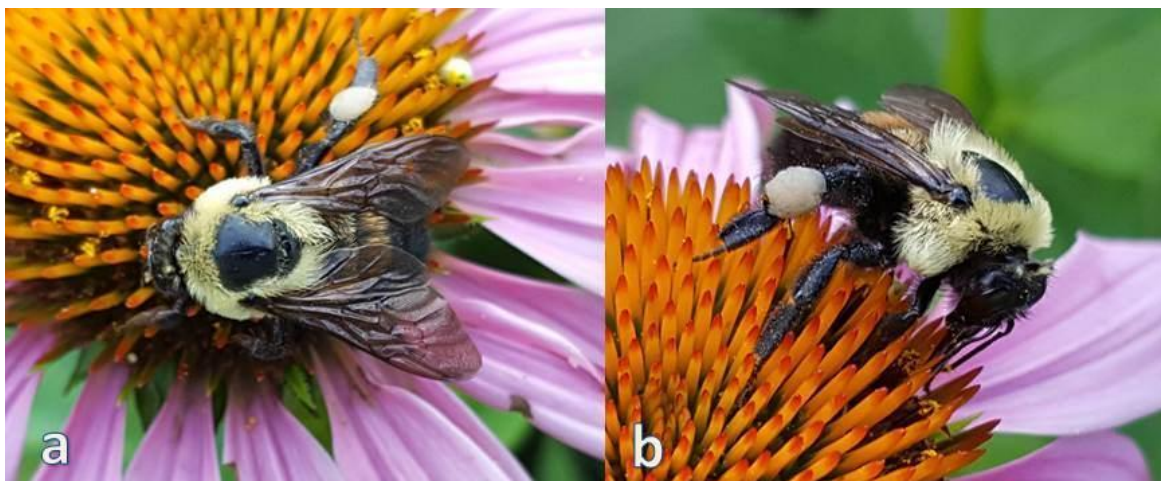


Figure C.3. Photographs of *B. griseocollis* (a) top view and (b) side view, which can be confused with *B. affinis*. Photographs were taken by Tamara Smith, USFWS.

Appendix D - How to Verify Records

- Self verification of species is encouraged and **Appendix C – Bee Identification** (above) provides useful information to help with identification.
- If verification/assistance with identification of potential *B. affinis* observations are requested, the surveyor acknowledges that data may be shared with third parties in order to accomplish this. The USFWS may submit by email the images and county level locations to a qualified expert for identification assistance, or if project information or locations are non-sensitive, the USFWS may submit the necessary information (including photos and locations) to other qualified experts (*e.g.*, BumbleBeeWatch.org). Once species identification has been made, the surveyor will be informed by USFWS of the results. If these requests require a significant amount of time and resources, changes to the protocol may be made.
- Proper documentation - verification requests to the lead field office should include geographic coordinates (decimal degrees) and photos that meet the standards in **Appendix A - Standardized Bee Photography**.

Appendix E - Further Information regarding Survey Effort

Based on a historical data set (Macfarlane 1974, in Colla and Packer 2008), if 150 *Bombus* were collected, the probability of missing *B. affinis* if it were present at historical abundance levels was 5%. In other words, if *B. affinis* is present at historical abundance levels, 150 bumble bees would need to be collected in order to detect the species with 95% confidence (Colla and Packer 2008, p. 1382). Recognizing the declines in abundance, capturing more individuals may be needed to reach this confidence level. We are unaware of similar detection probabilities for timed search surveys for bumble bees, however, based on expert input, we think it is reasonable that at least 1-person-hour of active sampling of 3 acres of high quality habitat, surveyed four times over the sampling season, will be sufficient effort to be reasonably certain that we would detect *B. affinis* if it were present. Other streamlined monitoring protocols recommend using timed transect searches (*e.g.*, Ward et al. 2014), similar to Pollard walks designed for butterfly monitoring (Pollard et al. 1993, entire) to estimate bee diversity and abundance, so we have suggested modified timed-area transect methods as one option for long-term monitoring. Whichever method is chosen, repeated sampling over multiple years will strengthen our confidence in species detections and non-detections. We hope the data collected through repeated surveys will help us develop detection probabilities of *B. affinis* and refine our survey recommendations to maximize efficiency and minimize uncertainty.

Appendix F - About Surveys for Proposed or Ongoing Actions that May Impact *B. affinis*

As a reminder, for those interested in seeking incidental take coverage through Section 7 or Section 10(a)(1)(B) of the Endangered Species Act, it is important to first review the Section 7 or 10 guidance

(<http://www.fws.gov/midwest/endangered/insects/rpbb/projectproponentsguidance.html>) to determine if the rusty patched bumble bee is likely to be present and surveys are recommended for such purposes. The Service considers the rusty patched bumble bee likely to be present only in “**high potential**” zones” as described online indicated in the Section 7 and 10(a)(1)(B) guidance (<https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>). This survey protocol document includes different voluntary survey protocols for a variety of purposes in high potential, low potential, and unoccupied zones (defined on our website <https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>).

To determine if *B. affinis* may be in an action area, action agencies or project proponents may screen projects online via the USFWS’s Information for Planning and Conservation website (IPaC, <https://ecos.fws.gov/ipac/>), or contact the USFWS Ecological Services Field Office for their area. If this screening indicates that a project is within an area where the rusty patched bumble bee is likely present (*i.e.*, the High Potential Zone), action agencies and others should coordinate with their state’s USFWS Ecological Services Field Office (FO). An online directory of USFWS FO(s) is available at <http://www.fws.gov/offices/>.

If a project action area is within an area where *B. affinis* is likely present (“High Potential Zones”, defined below, <https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html>), an action agency or project proponent may choose to conduct a survey to verify presence (note that negative surveys cannot verify absence with 100% confidence, but can at best predict likelihood of occupancy). The results of a survey, if they are negative and are carried out in accordance with FWS-recommended survey protocol (*i.e.*, Project Review Protocol), would indicate that the species would not be exposed to stressors associated with the action area. Project Review Protocol recommends one sampling season of surveys with sufficient effort⁹ to support a determination that the species is not likely present in the portion of the action area surveyed. Although not required for project review, repeat sampling events in the same areas (if still suitable) for at least 3 consecutive years are advisable if you are trying to be reasonably certain that the species no longer occupies the area.

⁹ Sufficient effort would consist of four approximately equally spaced sampling periods during the sampling season (early June to mid-August); one-person hour of search time per three acres of suitable high quality habitat (defined below) using non-lethal netting techniques. This document provides further details on methods, techniques, and best practices and is subject to continual improvement and modification.

Note that surveys should be conducted within a year before the project is initiated for negative survey results to remain valid throughout the duration of the project unless new information (*e.g.*, new positive surveys) suggests that the species is likely to be present in the action area. In that case, action agencies and the FWS field office (<https://www.fws.gov/offices/>) should work together to ensure that the best available information is considered.

If an action agency or project proponent chooses to conduct a survey to verify presence or absence, we recommend that they and their surveyors develop a proposed survey strategy in coordination with the USFWS FO(s) so that all parties fully understand which methods will be deployed, what assumptions will be made, and what the various outcomes would be based on the survey results. If not already required by federal permit, we recommend that survey results are submitted (negative or positive) to the USFWS FO(s) in the state(s) where the surveys took place. We strongly encourage this coordination as it improves the USFWS' understanding of (1) the level of survey effort underway and (2) the distribution of the species.