

An initiative of the Weeds Surveillance
Community of Practice, established
through the Canadian Plant Health
Council

Harmonized surveillance protocol for common waterhemp (*Amaranthus tuberculatus*) and other *Amaranthus* species

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Harmonized surveillance protocol for common waterhemp (*Amaranthus tuberculatus*) and other *Amaranthus* species

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1. Objective

Harmonization of surveillance protocols for *Amaranthus* species, specifically for common waterhemp (*Amaranthus tuberculatus*), for all Canadian provinces. This document can be used as a template for future hybrids weed surveillance efforts, including for *Amaranthus* hybrids.

2. Context

Early detection of non-native or recently introduced species to a region, such as common waterhemp and Palmer amaranth (*A. palmeri*), is crucial to establish a successful management program.

Palmer amaranth and common waterhemp are prolific and very competitive weeds that have biotypes resistant to multiple herbicide groups (up to 8 in the USA) (Sprague & Burns, 2021). These species are both dioecious, with plants having only male or only female flowers, with only the female plants producing seeds. At present, common waterhemp is reported only in Canadian fields in Ontario, Quebec and Manitoba. Other *Amaranthus* species present in Canada are monoecious (i.e. plants have both female and male flowers) and all can produce seeds. There are several monoecious *Amaranthus* species that are not native to Canada but are now widespread in many provinces including redroot pigweed (*A. retroflexus*), green pigweed,¹ (*A. powelli*), smooth pigweed (*A. hybridus*), and tumble pigweed (*A. albus*) (Weaver et al. 1980; Costea and Tardiff 2003; Costea et al. 2004; data.canadensys.net/vascan). Other amaranth species present in Canada include purple/livid amaranth (*A. blitum*) with limited distribution in Ontario and Quebec, and prostate pigweed (*A. blitoides*) which is not found in Atlantic Canada (Costea and Tardiff, 2003). Reported species also include spiny amaranth (*A. spinosus*) in Ontario and Manitoba, prince's-feather amaranth (*A. hypochondriacus*) in British Columbia, and foxtail amaranth (*A. caudatus*) in Quebec and Saskatchewan. The prince's feather amaranth, foxtail amaranth, and blood amaranth (*A. cruentus*) are cultivated for grain or as a forage elsewhere in the world, and there is potential to grow it in North America (Nurse et al. 2016; [Speciality Croppoportunities – Grain Amaranth \(gov.on.ca\)](https://www.gov.on.ca/agriculture/2016/05/specialty-croppoportunities-grain-amaranth)). A native species, the California amaranth (*A. californicus*), is present in Saskatchewan and Alberta (see data.canadensys.net/vascan).

¹ Also known as Powell's amaranth or green amaranth.

The present document proposes a common protocol for the surveillance of *Amaranthus* species, specifically for common waterhemp. Specific protocols have been developed in Quebec for monitoring waterhemp (early and late season detection, plus sampling for resistance) and are the basis of the present document (Mathieu et al., 2020a, Mathieu et al., 2020b, Mathieu et al., 2020c, Picard et al., 2021).

3. Biosecurity measures to prevent the spread of *Amaranthus tuberculatus*, non-native *Amaranthus* species and hybrids

Common waterhemp is not widely established across Canada. Therefore, it is important to adopt biosecurity measures to prevent the accidental spread of this species to other parts of the farm or other locations.

3.1. Materials

- Boot protectors or brush to clean boots before exiting the field (see boot cleaning kit)
- Protective clothing (overalls)² and head gear (hat, hair ties, etc.)
- Ziploc bags
- Dust/lint roller
- Bin or garbage bags to contain soiled clothing or boots
- Hairbrush
- Other items that may be useful:
 - boot cleaning kit (plastic bin, manual pressure pump filled with water, brush)
 - brush, scraper, small broom
 - hand cleaner and disinfectant, disposable gloves
 - vaporizer containing a cleaning agent
 - paper towels, wipes and linen
 - clean shoes for entering/driving the vehicle

3.2. Procedure

The following procedure is to be followed before and after scouting each field, to avoid transporting weed seeds and/or pollen from one field to another.

When entering the field

1. Make sure your clothes, vehicle and tools are clean before arriving to the farm.
2. Whenever possible, drive to the field using a vehicle belonging to the farm and not your own.
3. Park the vehicle on paved or gravel roads to avoid contaminating the vehicle as much as possible. Do not enter the field with the vehicle.
4. Put on your protective clothing. If applicable, tie your hair up and wear a hat.
5. Always wear boot protectors. Use a new pair of boot protectors for each field. If they are not available, use a brush to clean the soles of your boots before entering each field.

Before exiting the field

1. Brush your hair and shake your head gear (hat, cap, etc.).
2. Take off the protective clothing and shake it or clean it using a dust/lint roller. Put the (disposable) protective clothing in a garbage bag or plastic bin.
3. Remove the boot protectors just before entering the vehicle. If the boot protectors were not available, use a brush to clean the soles of your boots before exiting each field. If the boots are muddy, you could wash them using the *boot cleaning kit* (e.g. with the manual pressure pump)

² It is recommended that the scout wears protective clothing (i.e. overalls) and head gear once the amaranths have started flowering and/or producing seeds, in order to minimize the risk of transporting pollen and seeds in the clothing. A fabric overall is preferred, as the disposable ones may attract the pollen or the seeds due to static electricity.

to remove all traces of soil or plant material. Make sure to dispose of the water in the field but avoid pouring it on the edge of the field or into running water (to avoid transporting seeds to adjacent fields).

4. Place the used boot protectors in a garbage bag.
5. Make sure the vehicle (i.e. tires) is clean before leaving the farm.
6. Dispose of all the used material (boot protectors, disposable coveralls, etc.) in the garbage, using a double garbage bag.

Additional information

In French:

- [Trousse d'information - La biosécurité dans le secteur des grains](#)
 - La Fiche 7 (Lignes directrices pour les intervenants du secteur des grains)
 - Fiche 3A (Équipements et véhicules de transport – mesures à prendre)

In English:

- OMAFRA Farm Visit Biosecurity Protocol (see annex).

4. Identification of *Amaranthus* species

4.1. Traditional identification

Amaranth species are not easy to distinguish at the seedling stage as the differences in seedling morphology are subtle, and many are difficult to distinguish in the vegetative state. Genetic tests are recommended when plants are not in flower to identify species (see section 6: *Testing for herbicide resistance and species identification*). Redroot and smooth (not shown) have more pronounced pubescence (soft down or fine short hairs) compared to other amaranth species: expanding redroot pigweed leaves have some hairs at the margin and on the veins beneath while smooth pigweed leaves have hairs only on the margin of the leaf base (Kummer 1951). The stem of redroot will eventually be clearly pubescent (after the third leaf) and the stem of green pigweed will also be somewhat pubescent compared to waterhemp which is hairless (glabrous). Waterhemp seed leaves (cotyledons) are small (1.5-7.5 mm long) compared to other species (**Photo 1**). Most commonly, seedlings of common lamb's quarters (*Chenopodium album*) gets mistaken for *Amaranthus* seedlings, due to similar shaped cotyledons and first leaves (**Photo 2A**). Likewise seedlings of the Polygonaceae family including wild buckwheat (*Fallopia convolvulus*) (**Photo 2B**) and Lady's-thumb (*Persicaria lanigera*) (**Photo 2C**) can be easily mistaken for Amaranth seedlings due to their narrow cotyledons.

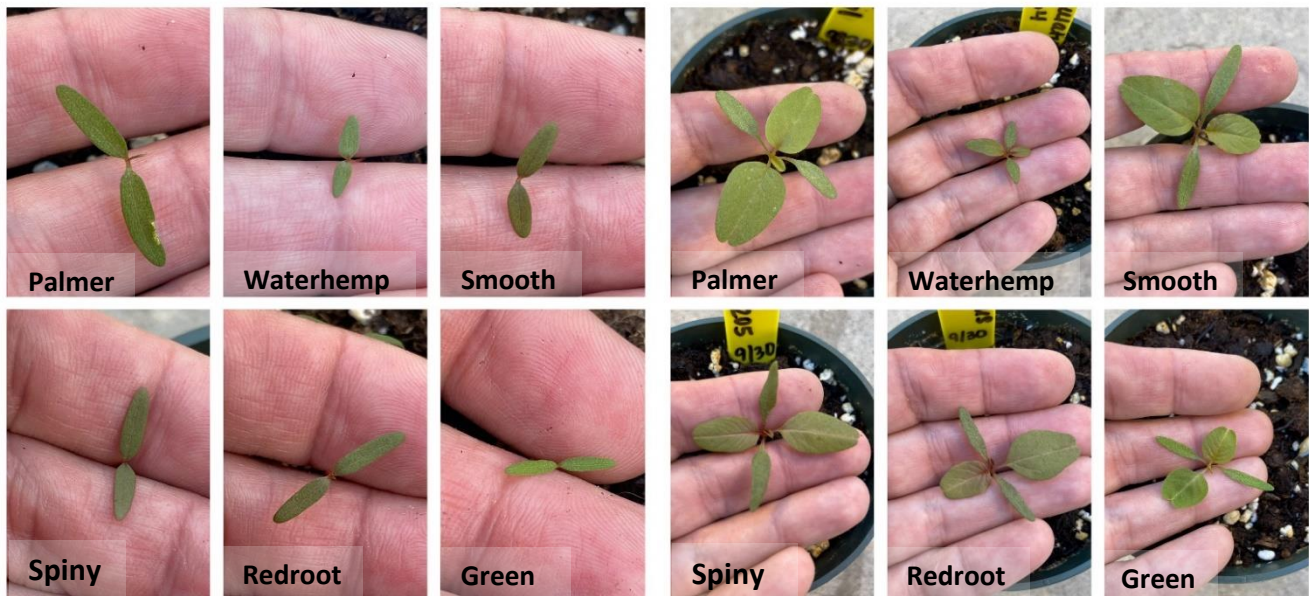


Photo 1. Pictures of six amaranth species found in Canada and the United States at the cotyledon to 2-leaf stage. (Photo credit: Dr. Lynn Sosnoskie, Cornell University)



Photo 2. Other seedlings that can be confused with common waterhemp. A) Common lamb's-quarters, B) Wild buckwheat, C) Lady's-thumb. Photo credit: MAPAQ, LEDP.

Other Resources for Identification:

- English: [Pigweed Species Identification Guide available | Grower \(thegrower.org\)](#)
- French : [Différenciation entre les espèces d'amarantes \(Réseau d'avertissements phytosanitaires\)](#)

4.2. Identification using molecular techniques

A molecular test for the identification of different species of *Amaranthus* is available in Ontario and Quebec. The procedure for taking the sample and sending it is described in section 6 (*Testing for herbicide resistance and species identification*).

5. Surveillance of *Amaranthus* species

5.1. Data collection

Scouting for common waterhemp is done to detect new infestation sites and test for herbicide resistance. Two scouting methods are presented in sections 5.2 (*Early surveillance of Amaranthus tuberculatus*) and 5.3 (*Late season surveillance of Amaranthus tuberculatus*).

The following information must be collected from each site regardless of when the scouting is done (early or late in the season) as this information will be useful when deciding the strategies of control that best suit each field:

a) Information about the site

- the region
- the municipality (of the field)
- the name of the farm
- the name of the producer
- the address (of the field)
- the field number
- the GPS coordinates of the field area and weed species if in patches
- crop - current year (indicate if the hybrid has technologies such as Roundup Ready, Liberty Link, etc.)
- soil texture
- previous crop
- tillage the previous fall
- tillage in spring
- crop planting date
- planting method (no-till or conventional)
- the herbicide treatment(s) applied in the current year
- the presence of a cover crop or intercropping
- the use of a contractor
- the presence of any other species of amaranth

b) Phenological stage of common waterhemp

Determining the phenological stage of the plant is helpful to determine: the emergence patterns (date of first emergence, as well as subsequent emergence flushes during the season); the window for chemical control (as most herbicides are less effective when the plants are taller than 10 cm); the window of opportunity to hand-weed to avoid seed production and/or collect seeds for herbicide testing.

The following phenological stages are proposed:

- Seedling: 2 true leaves or less.
- Vegetative < 10 cm: vegetative stage measuring less than 10 cm.
- Vegetative > 10 cm: vegetative stage measuring more than 10 cm.
- Vegetative > crop: vegetative stage taller than the crop (the type of crop must be stated).
- Flowering: stamens and/or pistils are visible.
- Seed bearing: presence of mature seeds.

As many cohorts at different phenological stages of the common waterhemp can be present at once, it is recommended that observations of the youngest stage, the most advanced stage and the predominant phenological stage are noted at each visit.

c) Distribution in the field and % infestation

- Both the field and the edge/entrance of the field must be scouted, to note the presence/absence of common waterhemp.
- If common waterhemp is present, then the general % of infestation must be noted (0% - 100 %). Alternatively, the % of infestation could be noted using the following classes:
 - 1: <1%,
 - 2: 1-5%,
 - 3: 6-25%,
 - 4: 26-50%,
 - 5: 51-75%,
 - 6: >75%
- Note the general distribution of the plants in the field: isolated plants, patches of plants, plants in rows, or randomly distributed plants.

5.2. Early season scouting of *Amaranthus tuberculatus*

Scouting early in the season allows for the implementation of control methods. The spread of infestations via farm machinery is a serious concern. Farms that do not have known infestations of common waterhemp but have recently bought and used imported machinery (e.g. second hand combine) or that share machinery that go through fields already infested with common waterhemp are particularly at risk. Early season scouting is recommended (i.e., at the beginning of the germination period and especially before the plants produce seed) for the fields at risk and/or the fields suspected of having amaranth infestation. Observations made in Ontario and Quebec suggest that common waterhemp could start emerging in late May or early June.

5.2.1. Materials

- Notepad, pencil, etc.
- Camera or cellular phone
- GPS or cellular phone (with GPS capabilities)
- Permanent marker
- Ziploc bags
- Pocket knife

5.2.2. Procedure

It is important to gather some preliminary information from the producer, to find out in which order the fields were harvested (using the newly imported or shared machinery) the previous fall, and where the machinery entered each field, etc.

Refer to section 4 (*Identification of amaranth species*) for additional information and resources available for the identification of common waterhemp and other amaranth species.

1. When scouting, pay particular attention to field entrances as well as ditch/water course edges.
2. Scout the edges of the ditches/water courses for the first 100 meters from the entrance of the field and for about 15 meters from the edge of the ditch/water course (See **Figure 1**). Walk through this area from top to bottom, separating each survey area by a few rows to determine if common waterhemp is present. Pay special attention to clumps/patches of seedlings.
3. If you find a common waterhemp plant or suspect you have found one:
 - a. Proceed to collect data on phenological stage, distribution and % of infestation, as indicated in section 5.1 (*Data collection*).
 - b. Register the location of the plant or patch using a GPS or a GPS application on your smartphone (e.g. Handy GPS application by BinaryEarth). Name the point: No. of field site 1 or 2 or 3, etc.
 - c. Take a few photos to document the plants observed in the field. Send the photos to the regional diagnostic lab or regional extension specialist to confirm the identification and await further instructions before sending the tissue samples or collecting seeds³.
 - d. Take tissue samples of the plant to confirm the species and test for resistance. Refer to section 6 (*Testing for herbicide resistance and species identification*) to see if the molecular test for resistance is available in your province and for the procedure to take the sample and send it for testing. If seeds are present, collect some following the procedure described in section 6.3 (*Sampling for herbicide resistance using bioassay testing via LEDP*) and send them to the laboratory for testing.
4. Once the presence of common waterhemp has been confirmed, you can share the data from the site (without any personal information) and/or learn more about the available control methods by contacting your regional diagnostic lab or regional extension specialist. See section 7 (*What to do if a new population of non-native Amaranthus species is detected*) for further details.

³ In Québec, the image can be sent to the LEDP to the address: mauvaiseherbe@mapaq.gouv.qc.ca. For more information consult the document: RAP-malherbologie, Avertissement No. 2, 17 mai 2021. <https://www.agrireseau.net/rap/documents/105598>

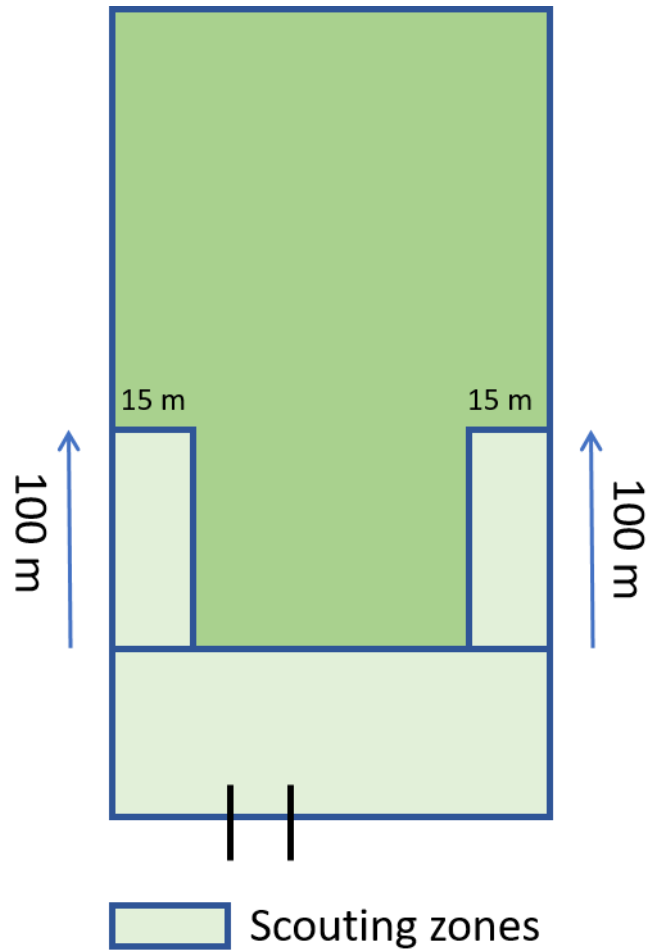


Figure 1. Scouting zones for common waterhemp in targeted fields. Adapted from : Mathieu, S. et Duval, B. 2020. Protocole de dépistage de l'amarante tuberculée - Printemps. Plan d'intervention phytosanitaire pour lutter contre l'amarante tuberculée. Coordination services-conseils (CSC). p. 5.

5.3. Late season scouting of *Amaranthus tuberculatus*

As mentioned above, farms that do not have known infestations of common waterhemp but have recently bought used imported machinery (e.g. second hand combine) or share machinery that go through fields already infested with common waterhemp are at a higher risk. Therefore, it is important to ask the grower which fields were harvested first, and where the machinery entered each field. This information may be useful to help pinpoint the places where common waterhemp is more likely to be present.

Surveillance done in late August and September could be complemented by taking samples of mature seeds and leaf tissue to test for resistance (see section 6.3 *Sampling for herbicide resistance tests*, for additional information).

5.3.1. Materials

- Notepad, pencil, etc.
- Camera or cellular phone
- GPS or cellular phone (with GPS capabilities)
- Permanent marker
- Ziploc bags
- Plastic bags
- Bucket or plastic bin
- Pocket knife

5.3.2. Procedure

1. Scouting can be done from mid-August until the end of September.
2. When scouting, pay particular attention to field entrances as well as to the edge of the ditch/water course.
3. Walk the field from top to bottom, separating each survey area by 10 meters to ensure the entire area has been surveyed (see **Figure 2**). Look over the entire field (field scan) and look for common waterhemp in the distance above the crop canopy (**Photo 3A**). Be aware that it is possible to find plants growing under the canopy (**Photo 3B**).
4. If you find a common waterhemp plant or suspect you have found one:
 - a. Proceed to collect data on phenological stage, distribution and % of infestation, as indicated in section 5.1 (*Data collection*).
 - b. Note the location of the plant or patch using a GPS or a GPS application on your smartphone (e.g. Handy GPS application by BinaryEarth). Name the point: No. of field_site 1 or 2 or 3, etc.
 - c. Take a few photos to document the plants observed in the field. Send the photos to the regional diagnostic lab or regional extension specialist to confirm the identification and await further instructions before sending the tissue samples or collecting seeds⁴.
 - d. Take tissue samples of the plant to confirm the species and test for resistance. Refer to section 6 (*Testing for herbicide resistance and species identification*) to see if the

⁴ In Québec, the image can be sent to the LEDP to the address: mauvaiseherbe@mapaq.gouv.qc.ca. For more information consult the document: RAP-malherbiologie, Avertissement No. 2, 17 mai 2021. <https://www.agrireseau.net/rap/documents/105598>

molecular test for resistance is available in your province and for the procedure to take the sample and send it for testing. If seeds are present, collect some following the procedure described in section 6.3 (*Sampling for herbicide resistance using bioassay testing via LEDP*) and send them to the laboratory for testing.

5. Once the presence of common waterhemp has been confirmed, you can share the data from the site (without any personal information) and/or learn more about the available control methods by contacting your regional diagnostic lab or regional extension specialist. See section 7 (*What to do if a new population of non-native *Amaranthus* species is detected*) for further details.

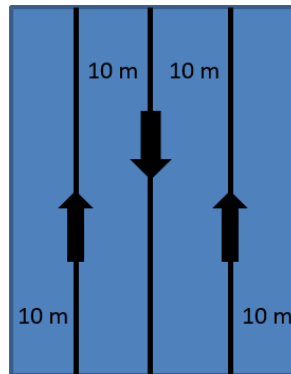


Figure 1. Scouting of the field in the fall. Adapted from: Mathieu, S. et coll. 2020. Dépistage de l'amarante tuberculée. Projet RAP- dépistage de l'amarante tuberculée. Réseau d'avertissements phytosanitaires (RAP) : p. 8.

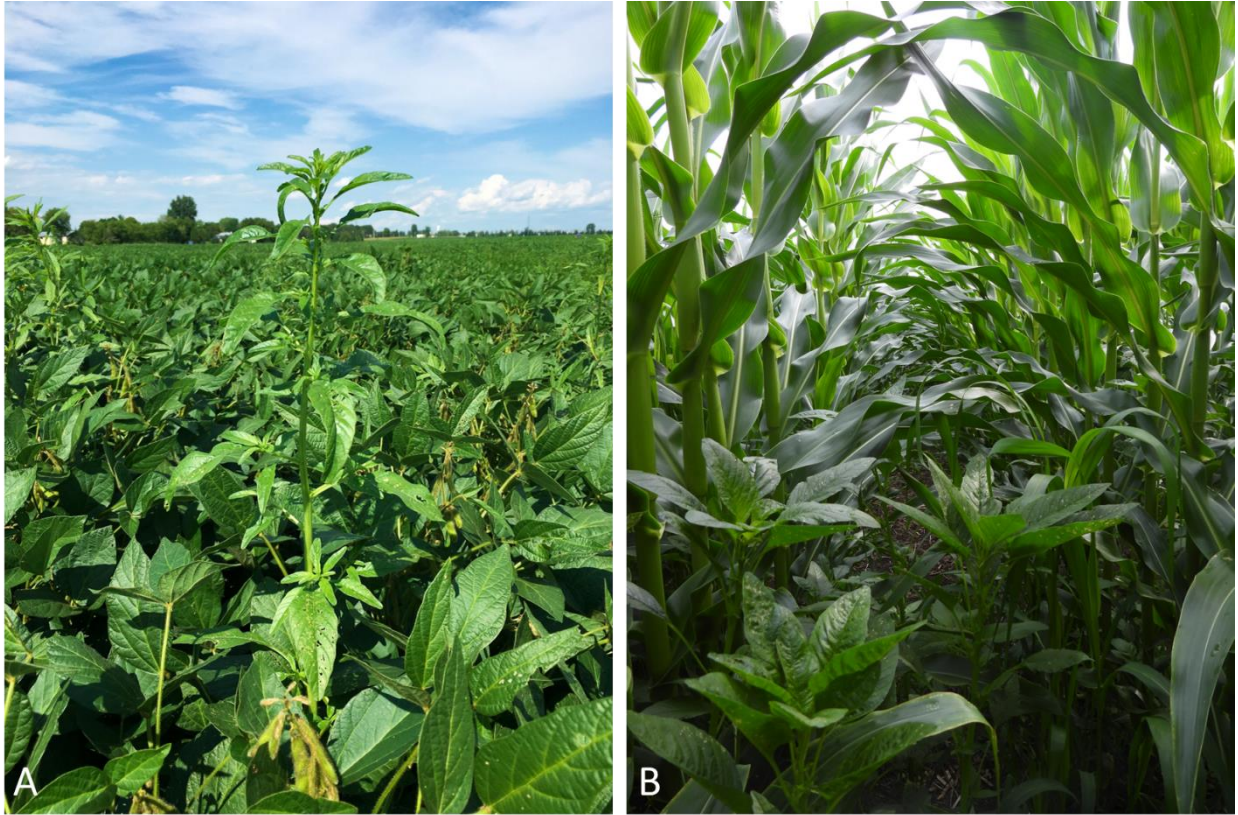


Photo 3. Common waterhemp in soybean (A) and under the canopy in corn (B). Photo credit: Sandra Flores-Mejia (CÉROM)



Photo 4. Common waterhemp in a greenhouse (A) and in soybean field (B). Photo credit: Marie-Josée Simard (A) and MAPAQ, Laboratoire d'expertise et de diagnostic en phytprotection (B)

6. Testing for herbicide resistance and species identification

6.1. Available tests and laboratories

There are two types of tests for detecting herbicide resistance:

1. Molecular tests - only available for some combinations of species and herbicide group resistance. Require relatively small amount of tissue (quarter-sized leaf tissue).
2. Bioassays - available for any weed species and herbicide group resistance - require mature seeds collected from the suspected population. Bioassays can take more than 6 months to complete.

6.1.1. In Ontario

In Ontario molecular tests only are done by Turn Key Genomics for combinations of species and herbicide groups listed in **Table 1**.

Turn Key Genomics
 c/o Chris Grainger
 34 Hill Trail
 Guelph, Ontario
 N1E 7C5 519-635-4470
chris@turnkeygenomics.ca
<https://turnkeygenomics.ca/>

Table 1. Available molecular tests for detecting resistance and species identification for *Amaranthus* species in Ontario. Technology licensed from AAFC.

Weed Species	Herbicide Group	Resistance & Tests
Redroot pigweed <i>A. retroflexus</i>	-	Species identification
	2	Target-site (S653N & W574L)
	5	Target-site (A251V, S264G**, V219I & F274L)
Green pigweed <i>A. powelli</i>	-	Species identification
	2	Target-site (S653N & W574L)
	5	Target-site (A251V, S264G**, V219I & F274L)
	14	Target-site (Δ G210 in PPX2L)
Waterhemp <i>A. tuberculatus</i>	-	Species identification
	2	Target-site (S653N & W574L)
	5	Target-site (A251V, S264G**, V219I & F274L)
	9	Target-site: EPSPS gene amplification
	14	Target-site (Δ G210 in PPX2L)

6.1.2. In Quebec

In Quebec molecular tests are offered by the MAPAQ Laboratoire d'expertise et de diagnostic en phytoprotection (LEDP) for combinations of species and herbicide groups listed in **Table 2**.

Laboratoire d'expertise et de diagnostic en phytoprotection (LEDP)
 Complexe scientifique,
 2700, rue Einstein, D.1.200h
 Québec (Québec) G1P 3W8
 Tél. : 418 643 5027, poste 2700
<https://www.mapaq.gouv.qc.ca/fr/Productions/md/Services/Pages/Formulairephytoprotection.aspx>
<https://cerom.qc.ca/services/service-de-detection.html>

The bioassays are completed in collaboration with the Centre de recherche sur les grains (CÉROM), with LEDP acting as a point of contact (contact information above).

Note:

1. LEDP is currently offering its services free of charge for species identification and detection of herbicide resistance for *Amaranthus* spp. in Quebec.
2. All samples sent to LEDP should be sent along with an analysis request available here.
3. Avoid sending samples on Fridays or around long holidays to prevent them from lying around in the post and withering away.

Table 2. Available molecular tests for detecting resistance and species identification for *Amaranthus* species in Quebec. Technology licensed from AAFC.

Weed Species	Herbicide Group	Resistance & Tests
Redroot pigweed <i>A. retroflexus</i>	-	Species identification
	2	W574L & S653N
	5	S264G, V219I, A251V et F274L
Green pigweed <i>A. powellii</i>	-	Species identification
	2	W574L & S653N
	5	S264G, V219I, A251V & F274L
Waterhemp <i>A. tuberculatus</i>	-	Species identification
	2	W574L & S653N
	5	S264G
	9	EPSPS gene amplification
	14	ΔG210 in PPX2L

6.2. Sampling for molecular herbicide resistance and species identification tests

For the molecular tests fresh plant tissue is collected.

a) Sample Collection Kits:

- 1 “Ziploc” type plastic freezer bags (27 cm X 27 cm)
- 10 “Ziploc” type plastic freezer bags (16.5 cm X 15 cm)
- 10 Unwaxed paper coin envelopes with labels indicating required information
- Silica gel beads (10 grams)
- Sampling instructions
- Field history – see section 5.1 (*Data collection*)

Contact Kristen Obeid: kristen.obeid@ontario.ca for sample collection kits.

b) Harvesting plant samples:⁵

- 1) Clearly label sample envelopes:
 - a) Grower name and address (if this cannot be provided, please ensure to include the GPS. Grower details are never released; all information is reported on a county level)
 - b) Field number/identification
 - c) Sampling date
 - d) GPS coordinates or any information that would be useful in identifying the plant samples (e.g. their average height) as well as the exact sampling location
 - e) Investigator’s name
- 2) Collect a piece of young leaf tissue, about the size of a quarter.
- 3) Place the leaf sample in an unsealed small paper envelope. Do not place samples from more than one plant in each envelope.
- 4) To determine the percentage of resistant plants in a field or in an area of the field, collect at least 10 samples (10 envelopes) per field, each taken from a different plant. Before determining where to sample look for hot spots in the field that cannot be explained by equipment failure or weather issues.
- 5) Place the paper coin envelope in the plastic bag containing the silica gel beads.
- 6) Seal the plastic bag as soon as possible, removing as much air as possible (airtight).
- 7) Keep the samples dry and cool and ship them for analysis as soon as possible. The plant material should be dried after 24 hours.
- 8) Shipping instructions: preferably ship leaves within 24 hours of harvesting in a cooler/Styrofoam container to protect against extreme temperatures; use fast delivery service.
- 9) For shipping addresses refer to Sections 6.1.1 (in Ontario) and 6.1.2 (in Quebec).

⁵ The complete procedure in French is available [here](#) (page 7, section “Envoi d’un échantillon de mauvaise herbe”).

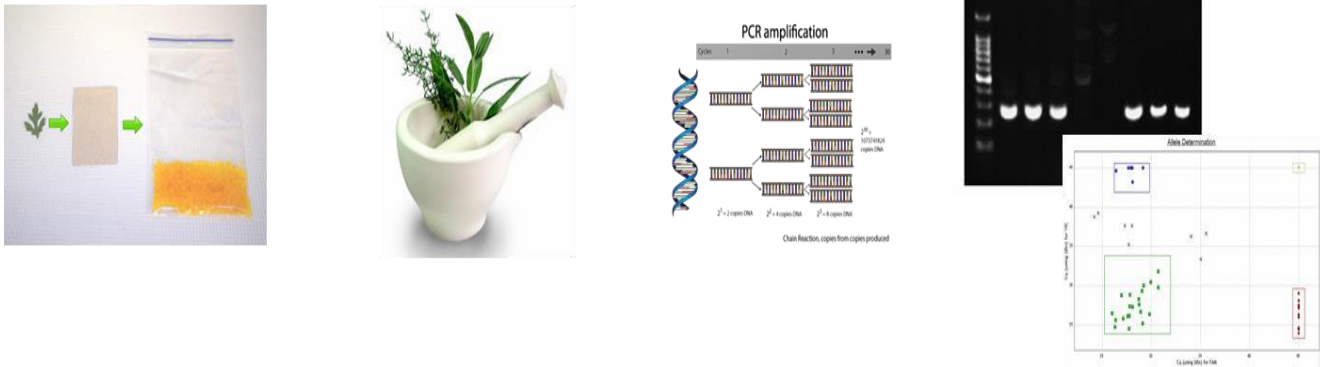


Photo 5. Example of a sample collection kit. Photo credit: Kristen Obeid (OMAFRA)

6.3. Sampling for herbicide resistance using bioassay testing via LEDP⁶

For the bioassays mature seeds are collected. In the case of common waterhemp, only the female plants produce seeds (**Photo 6**). Seeds are mature when they are hard.

- 1) Collect at least 1000 mature seeds from a minimum of 40 plants (will be equivalent to 500mL container). A similar amount of seed should be harvested from each of the plants sampled.
- 2) Roughly remove impurities, plant debris, insects or other debris that could affect the good preservation of the sample.
- 3) Place the seeds in a paper bag or envelope. Properly identify the samples by labeling them as in section 6.2.b.
- 4) Before sending the samples to the LEDP, store them in a dry place at room temperature. If necessary, spread the seeds on a large tray to dry them and prevent the formation of humidity.
- 5) Keep a record or map of the infested area.
- 6) Shipping instructions: seal seeds well against humidity and use a fast delivery service. Refer to Section 6.1.2 for the LEDP shipping address.

NOTE: The seeds should be sent to the LEDP along with an analysis request available [here](#) before December 1st of each year.

Tip for collecting waterhemp seeds:

Waterhemp is a dioecious plant, i.e. the male flowers (staminate flowers) and female flowers (pistillate flowers) are present on different plants. Identifying the flowers can be difficult due to their small size. Female flowers can be identified by the feathery styles from each ovary (**Photo 6B**). In comparison, the male flowers are more easily identifiable thanks to the presence of the pollen, (in the form of yellowish powder, **Photo 6A**). The seed is oval to elliptical in shape, slightly flattened and measuring 0.7 to 1 mm in diameter. It is present only in female plants and is inserted in an ovoid capsule (**Photo 6C**). The seed is dark brown to reddish brown in color, with a smooth surface.

⁶ The full procedure is available in French [here](#).

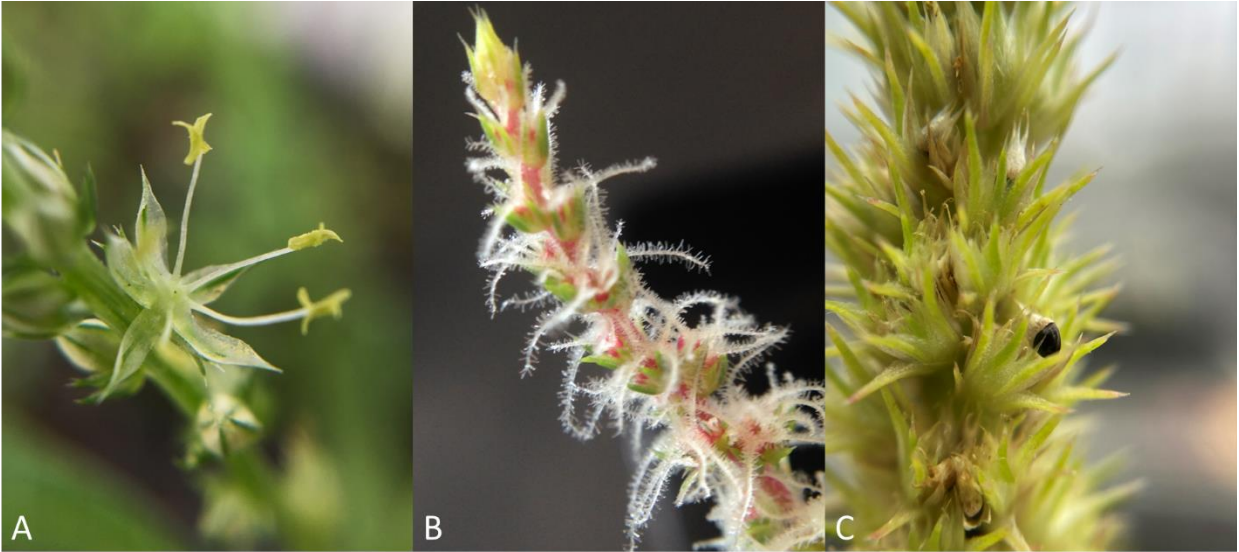


Photo 6. Photo of male (A) and female (B) flowers of waterhemp, as well of a mature seed on a female flower (C). Photo credit: Sandra Flores-Mejia (CÉROM).

7. What to do if a new population of non-native *Amaranthus* species is detected

If you think that you have found a plant/population of non-native *Amaranthus* species, contact the appropriate provincial specialist from the list below. In the absence of a provincial representative (i.e. for detections in BC, NL, NB), contact the Canadian Food Inspection Agency (CFIA) [here](#).

NOTE: If the detected plant/population is suspected to be Palmer amaranth, contact BOTH the provincial representative and the CFIA.

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8. Cited literature

1. Mathieu, S. *et al.* 2020a. Protocole de dépistage de l'amarante tuberculée - Printemps. Plan d'intervention phytosanitaire pour lutter contre l'amarante tuberculée. Coordination services-conseils (CSC). : p. 5.
2. Mathieu, S. *et al.* 2020b. Protocole de dépistage de l'amarante tuberculée - Automne. Plan d'intervention phytosanitaire pour lutter contre l'amarante tuberculée. Coordination services-conseils (CSC). : p. 5.
3. Mathieu, S. *et al.* 2020c. Dépistage de l'amarante tuberculée. Projet RAP- dépistage de l'amarante tuberculée. Réseau d'avertissements phytosanitaires (RAP). : p. 8.
4. Picard, A., et al. (2021). Réseau de surveillance de la croissance de l'amarante tuberculée. RAP-Malherbologie.
5. Kummer, A.P. 1951. Weed Seedlings. Chicago, University of Chicago Press 435 p.
6. Costea, M., Weaver, S., Tardif, F. 2004. The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. Can. J. Plant Sci. 84(2): 631-668.
7. Costea, M., Weaver, S.E., Tardif, F.J. 2005. The biology of invasive alien plants in Canada. 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif. Can. J. Plant Sci, 85: 507-522.
8. Costea, M., Tardif, F.J. 2003. The Biology of Canadian Weeds. 126. *Amaranthus albus* L., *A. blitoides* S. Watson and *A. blitum* L. Can. J. Plant Sci, 83: 1039-1066.
9. Weaver, S.E. McWilliams, E.L. 1980. The Biology of Canadian Weeds. 44. *Amaranthus retroflexus* L., *A. powellii* S. Wats. and *A. hybridus* L. Can. J. Plant Sci. 60: 1215-1234
10. Nurse, R.E., Obeid, K., Page, E.R. 2016. Optimal planting date, row width, and critical weed-free period for grain amaranth and quinoa grown in Ontario, Canada. Can. J. Plant Sci, 96(3): 360-366.
11. Sprague, C., Burns, E., 2021. Multiple herbicide-resistant Palmer amaranth & waterhemp in Michigan. *Michigan State University Weed Science*. Pp. 221-225; URL: <https://www.canr.msu.edu/weeds/extension/2022-Weed-Control-Guide/2022%20E-434%20Palmer%20&%20Waterhemp.pdf>

ANNEX:

**OMAFRA Farm Visit
Biosecurity Protocol**

FARM VISIT BIOSECURITY

for Visiting Orchards, Vineyards,
Crop Fields and Greenhouses

This handout outlines practices that can help minimize the risk of disease, insect, nematode or weed transmission when visiting orchards, vineyards, crop fields and greenhouses.

Additional biosecurity measures may be required in situations of a contagious plant disease or regulated crop pest. These may include protective (washable or disposable) footwear and clothing and enhanced disinfection procedures.

Discuss any concerns with your advisor where farm visit biosecurity requirements conflict with staff safety (e.g., wearing disposable clothing in high temperatures, wearing disposable footwear on slippery surfaces).

BASIC PROCEDURES:

Before the visit

- Call ahead to the grower, if possible,
 - Follow any additional protocol measures of the operation if applicable (e.g., for a regulated pest or contagious plant disease).
- Schedule field visits to avoid walking in wet fields or handling wet plants, if possible.
- Clean and disinfect any equipment to be used on plants.
- Wear clean clothes and footwear.

Upon arrival

- Drive slowly to avoid unnecessary contamination of vehicle (30 km/hr).
- Roll up the windows.
- Park in designated visitor parking or away from barns and other sources of contamination.
- Clean hands with disinfectant hand gel.
- Follow practices for tracking visitors (e.g., sign the visitor log book, send a text)
- Put on protective footwear and clothing before entering a field, orchard, vineyard or greenhouse, in high risk situations. Situations are high risk when a contagious plant disease or regulated pest is present. Wear protective footwear and clothing if unable to assess whether a situation is high risk.



Talk to your advisor about steps you should take when visiting farms this summer.

Follow a grower's biosecurity measures if more stringent than ours.

During the Visit

- If possible, leave vehicle in designated area and walk the crops. Limit driving through the fields as much as possible.
- Obey all signage and barriers. Do not enter buildings unless permitted by the farm operator.
- Avoid unnecessary direct contact with the crop. Certain diseases, such as tobacco mosaic virus, can easily spread by contact to other susceptible hosts.
- Wash and disinfect any equipment that will be used on plants between use on biologically separate and unique areas and/or building within the premises.

Before Leaving the Operation

- Visually inspect clothing, hair & equipment to ensure no insects or plant material are present or attached.
- Ensure footwear is free of excess soil and plant debris. Use a brush to remove soil from tread.
- Wash and disinfect footwear.
- Wash and disinfect any equipment used during the visit.
- Wash hands or clean hands with disinfectant hand gel.
- Leave disposable materials with the grower. Another option is to place disposable materials in a sealed plastic bag or washable sealable container in the vehicle for later disposal. Keep clean and dirty material separate.
- Inspect the interior and exterior of the vehicle for visible contamination (i.e. manure, insects, plant material and excess soil). Remove as much visible contamination as possible before leaving the operation. Wash contaminated vehicles before entering another agricultural operation.



ADDITIONAL MEASURES

When entering a protected structures (e.g., greenhouses, hoop houses)

- Disinfect hands using a germicidal lotion and/or wear disposable gloves.
- Wear clean shoes and clean clothes.
- Wear disposable or clean laundered clothing and disposable boots if coming into contact with the crop.

Where a known contagious disease or regulated crop pest is present

- Place any sample (plant, soil, insect or disease) taken at the facility, field, orchard or vineyard, in a container and seal so that the plant material or soil cannot inadvertently escape from the container.
- Visit the site(s) with known or suspected contagious and regulated plant pests last, if you have more than one site visit to do.
- Boots and field equipment **MUST** be cleaned with a disinfecting agent before leaving the site, if the contagious disease or regulated pest is soil-borne. Remove excessive organic matter from boots before disinfecting. Wash vehicle before entering another farm or facility.

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