Import of Palmer amaranth (*Amaranthus palmeri* S. Wats.) seed with sweet potato (*Ipomoea batatas* (L.) Lam) slips

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**Abstract:** Palmer amaranth is one of the most economically important and widespread weeds of arable land in the United States. Although no populations are currently known to exist in Canada, its distribution has expanded northward such that it is present in many of the states bordering Canada and multiple pathways exist for its introduction. In this short communication, we report on the transport of viable Palmer amaranth seed on imported sweet potato slips. A reproductive pair of Palmer amaranth seedlings were identified from soil accompanying imported sweet potato slips in 2018. Identification was confirmed using species-specific single nucleotide polymorphisms.

**Key words:** Palmer amaranth, sweet potato, import, barcoding.

**Résumé :** L’amarante de Palmer est une des adventices les plus répandues et les plus importantes, économiquement parlant, dans les terres arables des États-Unis. Bien qu’aucun peuplement n’ait actuellement été recensé au Canada, son aire a progressé vers le nord, au point qu’on en trouve désormais dans les États limitrophes et les voies ne manquent pas pour que l’amarante prenne pied au pays. Dans cette brève communication, les auteurs rapportent le transport de semences viables d’amarante de Palmer sur les boutures de patate douce importées. Deux plantules d’amarante de Palmer en mesure de se multiplier ont été retrouvées dans le sol qui enveloppait les boutures de patate douce importées en 2018. L’identification en a été confirmée grâce aux polymorphismes mononucléotidiques propres à l’espèce. [Traduit par la Rédaction]

**Mots-clés :** amarante de Palmer, patate douce, importation, code à barres.

**Introduction**

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is an annual dicotyledonous weed that is native to southwest United States and northwest Mexico (Sauer 1957). In his description of the Amaranths, Sauer (1957) noted that the genus “is notable mainly because of the success of many of its members as fellow-travelers of man-kind”. Indeed, in their recent review, Ward et al. (2013) describe how, over the past 20 years, Palmer amaranth has risen from relative obscurity to its current status as one of the most economically important and widespread weeds of arable land in the United States. Palmer amaranth can produce several hundred thousand seeds per plant and these tiny seeds (1–2 mm) are easily dispersed (Ward et al. 2013).
Evidence for the spread of Palmer amaranth along roadsides, in fields, and by birds have been offered up as important vectors of transport (Bagavathiannan and Norsworthy 2016; Farmer et al. 2017; Oseland et al. 2020), and its distribution and establishment has been facilitated by the evolution of resistance to multiple herbicide modes of action (Heap 2020).

The Canadian Food Inspection Agency (CFIA) and the United States Department of Agriculture (USDA) recently completed a joint weed risk assessment (WRA) of Palmer amaranth, each with separate written summaries (CFIA 2018; PPQ 2019). The CFIA–WRA document states that 14.5% of Canada is suitable for the growth and reproduction of Palmer amaranth. The CFIA lists Palmer amaranth as present in Canada, based on records from Ontario, but also acknowledges some uncertainty in its current status since its presence has not been confirmed recently. While we are unaware of any self-sustaining populations of Palmer amaranth in southern Ontario, there have been several specimens collected and stored in herbaria in this region. The WRA document lists three herbaria records in particular: (i) a collection by Gaiser in 1963 from Lambton Shores, Lambton County (Gaiser 3375F DAO), (ii) an undated University of Toronto record from St. Thomas, Elgin County (Scoggan 1979), and (iii) a 2007 collection by Oldham and Brinker from a disturbed site along railway tracks at a Niagara Falls railway yard, Niagara County, where it was observed to be well established and locally common (Oldham 34922 NHIC). Since the collection of the initial sample, this later site has been surveyed by the Ontario Ministry of Agricultural, Food and Rural Affairs (OMAFRA) and visited by local weed scientists, none of whom have observed any further evidence of Palmer amaranth (M. Cowbrough, personal communication).

It is clear that there are multiple pathways for Palmer amaranth to enter Canada including grain shipments, farm equipment, water courses, trains, horticulture transplants, nursery stocks, birds, and people. Palmer amaranth is currently listed as present in five states that border the Canadian province of Ontario (CFIA 2018; PPQ 2019), all of which have significant trade and infrastructure links with the province. The Canadian agricultural industry itself shares many linkages with the United States, some of which may represent significant vectors for the transport of weed seeds or propagules. For example, the production of sweet potato (Ipomoea batatas (L.) Lam) is reliant on horticultural propagules (i.e., slips) from regions of the United States where Palmer amaranth is widespread. Sweet potato slips are 25–30 cm long portions of vines growing from mother roots and are obtained by cutting vines at least 2–5 cm above the soil line. In 2016, sweet potatoes were grown on just over 700 ha across Canada, with the majority of production occurring in Ontario (Statscan 2016). It is currently estimated that at least ninety-five per cent of Ontario sweet potato producers purchase slips from the southern US, specifically North Carolina (M. Filotas, personal communication). Farmers in southern states are faced with managing Palmer amaranth biotypes that are resistant to up to six different herbicide modes of action: Groups 2, 3, 5, 9, 14, and 27 (HRAC 2020). While the import of sweet potato slips from the mid-South US is essential for the production of the crop in Canada, this practice also increases the risk of unintentionally importing Palmer amaranth. Given this potential, the objective of the following study was to (i) conduct an ad-hoc examination of shipments of sweet potato slips for seed of Palmer amaranth, and (ii) assess the herbicide resistance status of any viable individuals, should seed be discovered.

Materials and Methods

In 2018, four shipments of sweet potato slips originating from at least two different propagators in North Carolina were examined for the presence of accompanying soil. Sweet potato slips are shipped “soil-less” in cardboard boxes of approximately 1000, however, there is sometimes a small amount of soil at the bottom of the shipping container (Fig. 1). For each shipment, slips were removed from approximately 10–20 boxes and any remaining soil was shaken out of the box into a resealable bag. For two shipments, no soil was obtained from the boxes. For the remaining two shipments, <100 g of soil was obtained from each set of 10–20 boxes. The collected soil was mixed with commercial potting
soil (BM6, Berger, Saint Modest, Québec, Canada) in a 9”
tray and set out in a greenhouse with an alternating tem-
perature of 25 °C/20 °C. Soil was moistened daily to
encourage germination. Seeds that germinated and pro-
duced seedlings were identified, counted, and photo-
graphed for documentation. After eight weeks, the
trays of soil were moistened, placed in an opaque plastic
bag, and cold-stratified at 8 °C for six weeks. Following
stratification, the trays were returned to the same green-
house where they were monitored for emergence for an
additional four months. In 2019, soil was collected from
2 boxes of slips and treated as described above.

Two seedlings that emerged in 2018 were identified as
members of the Amaranthus genus and based on
taxonomic characteristics were visually identified as
Palmer amaranth. Leaf tissue was collected from both
individuals and genomic DNA was extracted using the
NucleoSpin Plant II kit (Machery-Nagel, Düren,
Germany) following the supplied protocol. The quantity
and quality of the DNA was determined by a NanoDrop
spectrophotometer and A260/280 ratios. Eluted DNA
was amplified by polymerase chain reaction (PCR) for
sequencing using primer sequences designed to amplify
the internal transcribed spacer (ITS) region. This region
of ribosomal DNA is widely used for plant barcoding
and contains single nucleotide polymorphisms (SNPs)
that have been shown to accurately distinguish
the many closely related Amaranthus spp. present in
North America (Murphy and Tranel 2018). Forward
(5′-TTTAGAGGAAAGGAGAAGTCGT-3′) and reverse pri-
mers (5′-CGTGGGCTCCTGTTTTAGG-3′) were designed
for the ITS region of Amaranthus tuberculatus (Moq.)
Sauer (Genbank accession no.KY968934.1). Primers were
synthesized by Integrated DNA technologies (Coralville,
IA) and PCR amplification was performed using 2× Taq
PCR Mastermix from Froggabio (230 Canarcit Drive,
Toronto, ON M3J 2X8, Canada). The cycling program
consisted of an initial denaturation at 95 °C for 1 min fol-
lowed by 35 cycles of 15 s at 95 °C, 15 s at an annealing
temperature of 56 °C, 30 s at 72 °C, and a final extension
of 7 mins at 72 °C. Sanger sequencing of the 562 bp PCR
product was carried out by the London Regional Genomics Centre. The resulting sequence data
was aligned using Sequencher software and examined
for previously reported target site mutations in ALS and
psbA (Beckie and Tardif 2012).

The Amaranthus seedlings were grown to physiologi-
cal maturity in a growth chamber with a photoperiod
of 14/10 h and a thermoperiod of 25 °C/20 °C. Mature
plants were re-examined with a focus on observing the
taxonomical characteristics of Palmer amaranth, sexed,
and dry mounted for preservation in the herbarium at
the Harrow Research and Development Centre. The
Amaranthus individuals were found to be male and
female, respectively, and were successfully crossed to
produced approximately 110 000 seeds.

Results and Discussion

A total of seven and eight seedlings emerged from the
soil that accompanied the sweet potato slips in 2018 and
2019, respectively. Two of the seedlings that emerged
from the 2018 sample were visually identified at the
seedling stage to be members of the Amaranthus genus
(Fig. 2). The remaining seedlings in 2018 were identified
as carpetweed (Mollugo verticillata L.) and sweet potato.
In 2019, four Canada fleabane (Conyza canadensis L.),
one carpetweed, one whitlow grass (Draba verna L.),
one common evening primrose (Oenothera biennis L.), and
one sedge (Carex ssp.) seedling were observed. Using single
nucleotide polymorphisms identified by Murphy
and Tranel (2018), the two Amaranthus seedlings from
2018 were positively identified as Palmer amaranth
(Table 1). Seedlings were grown to physiological maturity
and floral characteristics were used to further confirm
the identification.

This report represents the first evidence of transbor-
der transport of viable Palmer amaranth seed on horti-
cultural propagules. The introduction and potential
establishment of Palmer amaranth would represent a
serious challenge for the agricultural sector in Canada.
To date there are no reports of self-sustaining popula-
tions of Palmer amaranth in Canada, however, species
from the Amaranthus genus are very similar at the seed-
ling stage and it is conceivable that introductions of
Palmer amaranth have been misidentified as other
members of this genus.

Herbicide resistance traits could facilitate the establish-
ment of Palmer amaranth should seed be introduced
from a region where biotypes are known to possess resis-
tance. In the present study, the Palmer amaranth seed
came from North Carolina, where biotypes that have
single and multiple resistances to HRAC groups 2, 5, and
9 are presently described (Heap 2020). Results indicate
that neither of the individuals observed in this study

synthesized by Integrated DNA technologies and PCR
amplification was performed using 2× Taq PCR
Mastermix from Froggabio following the cycling condi-
tions described by Kohrt et al. (2017). Sanger sequencing
of the PCR product was again carried out by the London
Regional Genomics Centre. The resulting sequence data
was aligned using Sequencher software and examined
for previously reported target site mutations in ALS and
psbA (Beckie and Tardif 2012).
displayed any of the mutations known to confer resistance to groups 2 and 5 (data not presented). Unfortunately, we were not able to test for resistance to Group 9 herbicides, as known resistant and susceptible biotypes would have been required to assay EPSPS copy number, which is currently the only known resistance mechanism (Gaines et al. 2010). Based on these results, we can conclude that, had these individuals been introduced into an agricultural setting in Canada, they would likely have been controlled by herbicides commonly used in Ontario row crop and horticultural production systems. In sweet potato production in particular, herbicides from groups 3, 13, 14, and 15 are used in-crop for weed control (OMAFRA 2020), and the use of these herbicide modes of actions could have limited the potential for the survival and reproduction of these Palmer amaranth individuals. Indeed, the sweet potato fields where slips from this shipment had been planted were surveyed in August 2018, and one was surveyed again in 2019, and no Palmer amaranth was observed (M. Filotas, personal communication).

In summary, the results of this research represent the first report of the transport of viable Palmer amaranth seeds on horticultural propagules. Sweet potato slips have been imported from the United States into Canada for over 30 years and these results are the first observation of the transport of Palmer amaranth. While the two individuals observed in the present study were determined to be susceptible to herbicide from HRAC groups 2 and 5, the transport of multiple resistant biotypes of Palmer amaranth remains a possibility. To date, we are unaware of any observations of Palmer amaranth plants growing in Ontario sweet potato fields. Nevertheless, the Canadian agricultural community should remain vigilant, as the favourable climatic projections (CFIA 2018; PPQ 2019), proximity of Palmer amaranth populations in bordering states, and multiple avenues for entry into the country suggest that the survival and establishment of a self-sustaining population of Palmer amaranth in Canada may be only a matter of time.

Table 1. Amaranthus species-specific single-nucleotide polymorphisms (SNPs) adapted from Murphy and Tranel (2018). Numbers refer to nucleotide position within the internal transcribed spacer (ITS). Unknown samples represent the Amaranthus individuals collected from sweet potato slip boxes.

| Species       | GenBank Ref. No. | 28 | 58 | 63 | 69 | 73 | 83 | 108 | 141 |
|---------------|-----------------|----|----|----|----|----|----|-----|-----|
| A. hybridus   | KY968887.1      | T  | A  | T  | T  | A  | C  | C   | C   |
| A. retroflexus| KX079527.1      | T  | A  | T  | T  | A  | C  | C   | T   |
| A. powelli    | KX079503.1      | T  | A  | T  | T  | A  | C  | C   | T   |
| A. tuberculatus| KY968858.1      | T  | A  | T  | T  | G  | C  | C   | C   |
| A. virdis     | KC747451.1      | T  | G  | T  | T  | —  | C  | C   | C   |
| A. spinosus   | DQ005961.1      | T  | A  | T  | T  | —  | C  | C   | G   |
| A. albus      | JF973853.1      | T  | A  | C  | T  | —  | C  | C   | A   |
| A. palmeri    | KF493788.1      | A  | A  | T  | T  | —  | C  | A   | G   |
| Unknown 1     | N/A             | A  | A  | T  | T  | —  | C  | A   | G   |
| Unknown 2     | N/A             | A  | A  | T  | T  | —  | C  | A   | G   |

Note: Em dash (—) indicates a gap in the sequence.
Acknowledgements

The authors are grateful for the funding provided in support of this project by Agriculture and Agri-Food Canada (Project#J-001751) and the assistance of M. Bagavathiannan and B. Caldbeck.

References

Bagavathiannan, M.V., and Norsworthy, J.K. 2016. Multiple-herbicide resistance is widespread in roadside Palmer amaranth populations. PLoS ONE, 11: e0148748–e01487489. doi:10.1371/journal.pone.0148748. PMID:27071064.

Beckie, H.J., and Tardif, F.J. 2012. Herbicide cross resistance in weeds. Crop Prot. 35: 15–28. doi:10.1016/j.cropro.2011.12.018.

CFIA. 2018. Weed risk assessment for *Amaranthus palmeri* (Amaranthaceae) – Palmer amaranth Pages 32 in Plant Health Risk Assessment Unit, Plant Health Science Division. Plant Health Risk Assessment Unit, Plant Health Science Division, Ottawa, ON.

Farmer, J.A., Webb, E.B., Pierce, R.A., and Bradley, K.W. 2017. Evaluating the potential for weed seed dispersal based on waterfowl consumption and seed viability. Pest Manage. Sci. 73: 2592–2603. doi:10.1002/ps.4710.

Gaines, T.A., Zhang, W., Wang, D., Bukun, B., Chisholm, S.T., Shaner, D.L., et al. 2010. Gene amplification confers glyphosate resistance in Amaranthus palmeri. Proc. Natl. Acad. Sci. 107: 1029–1034.

Heap, I. 2020. The International Survey of Herbicide Resistant Weeds. [Online]. Available from http://weedscience.org/ [19 June 2020].

HRAC. 2020. HRAC mode of action classification 2020. [Online]. Available from https://hracglobal.com/tools/hrac-mode-of-action-classification-2020-map [5 May 2020].

Kohrt, J.R., Sprague, C.L., Nadakuduti, S.S., and Douches, D.J.W.S. 2017. Confirmation of a three-way (glyphosate, ALS, and atrazine) herbicide-resistant population of Palmer amaranth (*Amaranthus palmeri*) in Michigan. Weed Sci. 65: 327–338. doi:10.1017/wsc.2017.2.

Murphy, B.P., and Tranel, P.J.J.C.S. 2018. Identification and validation of Amaranthus species-specific SNPs within the ITS region: applications in quantitative species identification. Crop Sci. 58: 304–311. doi:10.2135/cropsci2017.06.0359.

OMAFRA. 2020. Guide to Weed Control: Hort Crops – Publication 75b. Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON.

Oseland, E., Bish, M., Spinka, C., Bradley, K.J.I.P.S., and Management. 2020. Examination of commercially available bird feed for weed seed contaminants. Invasive Plant Sci. Manage. 13: 14–22. doi:10.1017/inp.2020.2.

PPQ. 2019. Weed risk assessment for *Amaranthus palmeri* (Amaranthaceae) – Palmer amaranth. United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (PPQ), Raleigh, NC. p. 28.

Sauer, J. 1957. Recent migration and evolution of the dioecious amaranths. Evolution, 11: 11–31. doi:10.1111/j.1558-5646.1957.tb02872.x.

Scoggan, H. 1979. Flora of Canada. National Museums of Canada, Ottawa, ON.

Statscan. 2016. Census of Agriculture. Statistics Canada, Ottawa, ON.

Ward, S.M., Webster, T.M., and Steckel, L.E. 2013. Palmer amaranth (*Amaranthus palmeri*): a review. Weed Technol. 27: 12–27. doi:10.1614/WT-D-12-00113.1.