

ARTICLE

Received 15 Nov 2014 | Accepted 9 Jan 2015 | Published 9 Feb 2015 | Updated 25 Jun 2018

DOI: 10.1038/ncomms7262

OPEN

Thermogenesis-triggered seed dispersal in dwarf mistletoe

Rolena A.J. deBruyn^{1,†}, Mark Paetkau², Kelly A. Ross³, David V. Godfrey³, John S. Church⁴
& Cynthia Ross Friedman¹

Lodgepole pine dwarf mistletoe (DM), *Arceuthobium americanum*, is a parasitic flowering plant and forest pathogen in North America. Seed dispersal in DM occurs by explosive discharge. Notably, slight warming of ripe DM fruit in the laboratory can trigger explosions. Previously, we showed that alternative oxidase, a protein involved in endogenous heat production (thermogenesis) in plants, is present in DM fruit. These observations have led us to investigate if thermogenesis induces discharge. Here, infrared thermographs reveal that ripe DM fruits display an anomalous increase in surface temperature by an average of $2.1 \pm 0.8^\circ\text{C}$ over an average time of $103 \pm 29\text{ s}$ ($n=9$, 95% confidence interval) before dehiscence. Furthermore, both non-isothermal and isothermal modulated differential scanning calorimetry consistently show an exothermic event ($\sim 1\text{ J g}^{-1}$) in the non-reversible heat flow just prior to discharge. These results support thermogenesis-triggered seed discharge, never before observed in any plant.

¹Department of Biological Sciences, Thompson Rivers University, Kamloops, British Columbia, Canada V2C 0C8. ²Department of Physical Sciences (Physics), Thompson Rivers University, Kamloops, British Columbia, Canada V2C 0C8. ³Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, British Columbia, Canada V0H 1Z0. ⁴Department of Natural Resource Science, Thompson Rivers University, Kamloops, British Columbia, Canada V2C 0C8. †Present Address: Department of Occupational Therapy, University of Alberta, Edmonton, Alberta, Canada T6G 2G4. Correspondence and requests for materials should be addressed to C.R.F. (email: cross@tru.ca).

Dwarf mistletoes (DMs) belong to the genus *Arceuthobium* (family Santalaceae), and comprise a group of new and old world flowering plants that are aerial parasites on Pinaceae and Cupressaceae¹. These parasites are economically important in Canada, especially in British Columbia (BC) and Alberta, where they are destructive pathogens of commercially valuable coniferous timber trees. Infestation by DM is detrimental to host wood quality, impedes the trees' ability to resist other infestation and decreases the lifespan of infected trees. Currently, six *Arceuthobium* taxa have ranges that extend over nearly three quarters of BC, equating to a potential loss of >3.7 million m³ of coniferous forest a year². *Arceuthobium americanum* Nutt. ex Engelm., the American or lodgepole pine DM, commonly infects lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) in BC, where it is of considerable concern to the forest industry in that province.

Seed dispersal in DM is accomplished by a remarkable explosive process. DM spread solely by seed, which is forcibly ejected from the ripe single-seeded fruit at the end of the growing season¹ (late August to early September in BC). For a given stand of infected pines, dispersal takes place over the period of about a week. Ripe fruit are broadly fusiform-spheric, bicoloured and borne on short, recurved pedicels in groups of three or more on branching infructescences (Fig. 1). Seminal studies by Hinds *et al.*³ showed that a seed can be dispersed as far as 20 m from its source, with initial velocities approaching 25 ms⁻¹ (100 km h⁻¹). Viscin tissue, a mucilaginous region that forms a layer around the single seed within each fruit, accumulates the hydrostatic force needed for water-driven explosive discharge^{1,4}. Dispersal is ultimately achieved by explosive fracture in the abscission layer at the pedicel³⁻⁵. A somewhat analogous strategy for seed dispersal has been described for other plants, such as *Echium elaterium* (L.) A. Rich. (Mediterranean squirting cucumber), in which a hydrostatic pressure is also generated within the fruit⁶. *Hura crepitans* L. (Monkey's dinner bell)



Figure 1 | Ripe dwarf mistletoe (DM) fruit. DM fruit collected at the end of the growing season (early September in British Columbia) are broadly fusiform-spheric, bicoloured and borne on short, recurved pedicels in groups of three or more on branching infructescences. Discharge of the seed will ultimately occur at the abscission layer, located where the pedicel contacts the fruit. Scale bar, 4 mm. A version of this photograph was originally published in a paper by Kelly *et al.*¹⁷

similarly undergoes explosive fracture and water-related movement during seed dispersal, although differential drying rather than hydrostatic pressure drives the discharge in this species⁷.

To study explosive discharge of DM fruit in the laboratory, Hinds *et al.*³ were able to initiate dehiscence by slightly warming ripe fruit. While we cannot guess what these authors might have thought about the phenomenon, perhaps like us, they began to wonder if endogenous heat production (thermogenesis) might play a role in explosive discharge. Thermogenesis is the internal maintenance or increase of tissue temperature⁸⁻¹⁰. In plants, thermogenesis is most often accomplished through mitochondrial respiratory pathways that involve a cyanide-resistant alternative oxidase (AOX)¹¹. In contrast to the familiar respiration pathways that store energy by creating a proton gradient to generate ATP, AOX pathways create energy and release it as heat⁹. Ross Friedman *et al.*¹² found that the timing of explosive seed discharge in DM corresponds to the appearance of AOX in DM fruit mitochondria, which suggests that if there is a proper supply of substrate (for example, reduced ubiquinone) and a stable metabolic flow towards the mitochondria (including a supply of NADH), thermogenesis might induce seed dispersal.

Some flowering plants are known to employ thermogenesis for reproduction. For example, it is widely believed that the heat produced by thermogenesis in some Araceae volatilizes chemicals that attract pollinators to the flowers⁹. It is, therefore, not unreasonable to think that thermogenesis might play other roles in reproduction, such as in seed dispersal.

In our current study, infrared thermography (IRT) along with both non-isothermal (ramped temperature) and isothermal (constant temperature) modulated differential scanning calorimetry (MDSC) is used to detect endogenous heat production in ripe DM fruit before dehiscence to see if thermogenesis could indeed be occurring and possibly even inducing discharge. An average endogenous heat release of $\sim 1 \text{ J g}^{-1}$ via MDSC and an associated surface temperature increase of $\sim 2^\circ\text{C}$ from IRT in the minutes leading up to dispersal is identified in this study, supporting our hypothesis that thermogenesis may play a key role in explosive seed discharge in DM.

Results

Infrared thermography. All ripe test fruit examined ($n = 9$) with IRT displayed an anomalous positive surface temperature increase that initiated a short time (Δt) before dehiscence. An example of the anomalous increase in fruit surface temperature can be seen in Fig. 2, which depicts the time series of the temperature difference $T_{\text{test}} - T_{\text{control}}$. Early in the time series a linear increase is noted, indicating the test fruit is heating slightly faster than the control. In a perfect experiment, the control and test fruits would be identical in shape and size, and thus heat at the same rate so that the difference $T_{\text{test}} - T_{\text{control}}$ would be constant; however, no two fruit can be identically shaped or sized, and so there is a slight difference in the rates (see raw data in Fig. 2 inset) leading to a non-zero slope in the time series before 5.5 min. In the event shown in Fig. 2, the temperature difference anomaly starts at about 5.5 min; then, 1.5 min later, dehiscence occurs. By fitting the data from the 3- to 5.5-min period to a linear trend line, the temperature increase at dehiscence (ΔT) and the duration time of the anomaly (Δt) can be measured and used to describe the anomaly; that is, the anomaly results in an increase in temperature of ΔT and it commences Δt before dehiscence. The values and 95% confidence intervals ($n = 9$) for these two parameters are: $\Delta T = 2.1 \pm 0.8^\circ\text{C}$ and $\Delta t = 103 \pm 29 \text{ s}$. The anomalous temperature increase occurring just before dehiscence is evidence of thermogenesis. It is reasonable to expect that DM should

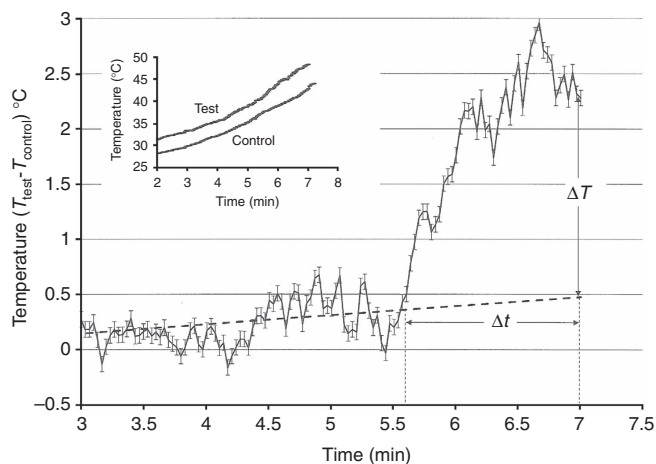


Figure 2 | Time series plot of temperature difference

($\Delta T = T_{\text{test}} - T_{\text{control}}$). In these representative data, dehiscence occurs near 7 min; 90 s prior, the temperature of the fruit begins to increase more rapidly than the control. The straight dashed line is the best linear fit to the data prior to 5.5 min, and extrapolation of this line defines ΔT and Δt . For this exploding (test) fruit, $\Delta T \sim 2.2^\circ\text{C}$ and $\Delta t \sim 90\text{ s}$. The inset (upper left) shows the raw data of the exploding and control fruit. The main graph has been shifted down 3.2°C for clarity. Here error bars (tolerance) are for temperature ($\pm 0.05^\circ\text{C}$), and are obtained from the infrared camera's temperature sensitivity of $\leq 0.05^\circ\text{C}$. This sample is one of nine trials.

undergo some ambient heating in the field similar to that which it experienced during the IRT experiments: plant leaf surface temperatures as high as 51°C can be reached during a sunny day¹³. The existence of ambient heating, though, does not negate the evidence for thermogenesis.

Modulated differential scanning calorimetry. Results from both non-isothermal and isothermal MDSC confirm that thermogenesis occurs prior to discharge. Figure 3 depicts the MDSC data and shows a representative non-isothermal run (circles) as well as a representative isothermal run (diamonds). The non-isothermal data correspond to the upper horizontal axis (temperature in $^\circ\text{C}$); the open circles track the reversible heat flow data, while the filled circles show the non-reversible heat flow. In the non-isothermal data, both reversible and non-reversible heat flow is constant until about 31.75°C ; at 31.75°C , the reversible heat flow begins to decrease (associated with wax and polysaccharide melt, an endothermic event), while the non-reversible heat flow begins to increase (an exothermic event). This increase in the non-reversible heat flow indicates thermogenesis. These trends in both the reversible and non-reversible flow continue until the explosion occurs at 32.4°C , which is characterized by a minimum in the reversible data and a large drop in the non-reversible data. Again, heating in the field could occur and be similar to that experienced in the non-isothermal DSC experiment, but this ambient heating, though, does not invalidate the consistent observation of thermogenesis.

Isothermal MDSC experiments performed at 25, 37 or 50°C were used to verify that the thermogenic event would occur in the absence of temperature ramping. All isothermal experiments showed an exothermic event before dehiscence, indicative of thermogenesis. The timing of the onset of exothermic event varied from ~ 1.2 to 9 min, and no trend between isothermal test temperature and the timing of exothermic event was evident. Dehiscence, noted by the onset of the endothermic event and occurring after the exothermic event, took place from ~ 1.8 to 10 min. Figure 3 depicts a representative thermogram showing

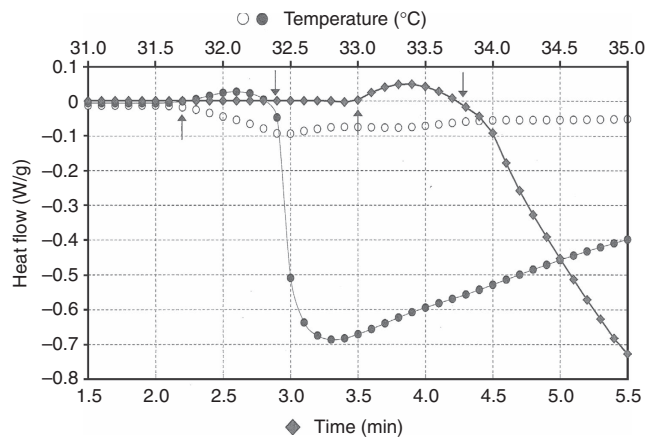


Figure 3 | Representative MDSC data. Circles represent non-isothermal (scanning) data and correspond to the upper horizontal scale, temperature in $^\circ\text{C}$. The open circles (○) are reversible heat flow data and the filled circles (●) are non-reversible heat flow data. The non-isothermal heat flows show local minima at about 32.4°C (down arrow), which are associated with dehiscence, and the dips in both data suggest a large endothermic event. Just before dehiscence, however, there is a small positive peak in the non-reversible heat flow data (starting at the up arrow), which is associated with an exothermic event. The filled diamonds (◆) represent the isothermal data and correspond to the lower horizontal scale, time in minutes. Again there is a small release of energy in the region indicated by the up and down arrows.

non-reversible heat flow (diamonds) under isothermal conditions ($T = 37^\circ\text{C}$); isothermal MDSC only reports changes in non-reversible heat flow data. The isothermal data correspond to the lower horizontal axis (time in minutes). The behaviour of the isothermal non-reversible heat flow is very similar to that for the non-isothermal runs. As in the non-isothermal case, the non-reversible heat flow for the isothermal test shown in Fig. 3 remains constant until ~ 1.2 min, when it then begins to increase (exothermic, thermogenesis). The fact that exothermic events such as the ones depicted in Fig. 3 are evident in data in the isothermal experiments performed at different temperatures (25, 37 and 50°C) and that the isotherms occur over a range of times shows that the exothermic behaviour is independent of the heating rate and isotherm temperature and thus, the isotherm data confirm that the exothermic event is not an artifact of heating the DM.

Thermogenesis. Using a nominal pre-explosion specific heat from the MDSC data of $0.6 \pm 0.4 \text{ J } ^\circ\text{C}^{-1} \text{ g}^{-1}$ ($n = 9$, s.d.) for the DM fruit, the energy per gram required for a temperature increase of $\Delta T \sim 2^\circ\text{C}$ as measured by IRT is $\sim 1.2 \pm 0.8 \text{ J g}^{-1}$ ($n = 9$, s.d.). Quantitatively, the exothermic events detected with non-isothermal MDSC experiments released an average heat of $0.77 \pm 0.08 \text{ J g}^{-1}$, or roughly 1% of the total (endothermic and exothermic) heating, while the exothermic events detected with isothermal MDSC experiments released an average heat of $1.25 \pm 0.79 \text{ J g}^{-1}$, or roughly 1.5% of the total (endothermic and exothermic) heating. The values obtained through IRT, non-isothermal MDSC, and isothermal MDSC are all within the bounds of experimental uncertainty, providing support for the idea that all of the experiments are examining related events.

Discussion

Given that the IRT experiments and MDSC experiments approach the measurement of thermogenesis differently—and

that non-isothermal and isothermal MDSC are quite different in how they measure heat flow—the fact that we obtained similar values for heat flow supports the assertion that all experiments are measuring a real and consistent thermogenic event. In the field, during the week or so over which dispersal occurs, ambient temperatures can slowly rise throughout the day (as mimicked by our non-isothermal tests), but can also remain constant for hours at a time (as reproduced in our isothermal tests). Therefore, while the thermogenic event manifested under both types of laboratory conditions, we need to do more work in the field to try to determine the link between ambient heating and endogenous warming, if any.

Work by Ross Friedman *et al.*¹² indicated that AOX in mitochondria show increased expression during fruit development. In many plants, heat is generated in the mitochondria as a secondary process of cellular respiration, and is enabled by electron transport chain AOX¹¹. Ross Friedman *et al.*¹² also thought that plastid terminal oxidases (PTOX), which became labelled with anti-AOX antibodies in maturing fruit plastids, might even have some role in thermogenesis. The possible function of PTOX in thermogenesis—if any—is less clear, but discovery of its involvement in thermogenesis might be novel. Future studies of thermogenesis in DM should endeavour to identify the molecular basis of thermogenesis to determine if there is a stable metabolic flow of substrates towards the mitochondria and/or plastids in DM fruit. These studies should be coupled with those in which AOX and PTOX gene expression and protein synthesis is monitored in DM fruits during their development.

In conclusion, this study provides compelling evidence that thermogenesis triggers explosive seed discharge in DM, a novel finding with respect to seed dispersal. Findings of an average endogenous heat release of $\sim 1 \text{ J g}^{-1}$ via MDSC and an associated surface temperature increase of $\sim 2^\circ\text{C}$ from IRT in the minutes leading up to dispersal support this conclusion. The data show that dehiscence is preceded by a thermogenic event. A logical conclusion is that endogenous heat production acts as an internal trigger for the final explosive fracture of the fruit at the abscission layer in the pedicel.

Methods

Infrared thermography. Groups of ripe DM (*Arceuthobium americanum*) fruit ($n = 9$ groups of 2–3 fruits) were collected from a stand of infected lodgepole pine (*Pinus contorta* var. *latifolia*) near Kamloops, BC ($50^\circ 31'$ latitude and $120^\circ 28'$ longitude) in early September 2013, and placed into a chamber in which the heat was increased at a rate of $\sim 2^\circ\text{C min}^{-1}$. A sample size of nine would readily be able to show real differences in temperature, as temperature anomalies should truly never occur randomly. Average surface temperatures of all DM fruit in the chamber (Fig. 4) were measured using a FLIR E60 infrared camera (thermal sensitivity: $< 0.05^\circ\text{C}$, 2% accuracy) and recorded as a time series using FLIR ThermaCAM software (Version 4.0.13284.103). The fruit that exploded first was deemed the test fruit, and its time series was referred to as T_{test} , whereas an adjacent fruit that did not explode within the same group in the chamber was called the control fruit, and its time series was termed T_{control} . It is possible to use an unexploded fruit as a control, since fruit will explode at different temperatures and therefore at various times, presumably due to different states of ripeness. As will be shown, anomalous heating occurs 1–2 min before dehiscence, and any fruit chosen as a control exploded much later (> 3 min) than the test fruit.

Since we wished to detect internal heating, the time series T_{test} was compared with T_{control} . The $T_{\text{test}} - T_{\text{control}}$ data were plotted versus time. If no endogenous heat is generated, plots of $T_{\text{test}} - T_{\text{control}}$ should be constant over time. Endogenous heat production manifests as a positive deviation of the temperature difference. Confidence intervals for the temperature difference and trend lines were calculated using Excel 2007 (Microsoft).

Modulated differential scanning calorimetry. A differential scanning calorimeter (2910 modulated refrigerated differential scanning calorimeter, New Castle, Delaware) was used to generate thermograms from DM fruit $17.0 \pm 3.1 \text{ mg}$ ($n = 42$, s.d.) collected from the infected pine stand (described in previous section) during the first week of September in both 2013 and 2014. Again, a bona fide heat increase should not be a random occurrence, so our sample size of 42 is large enough to

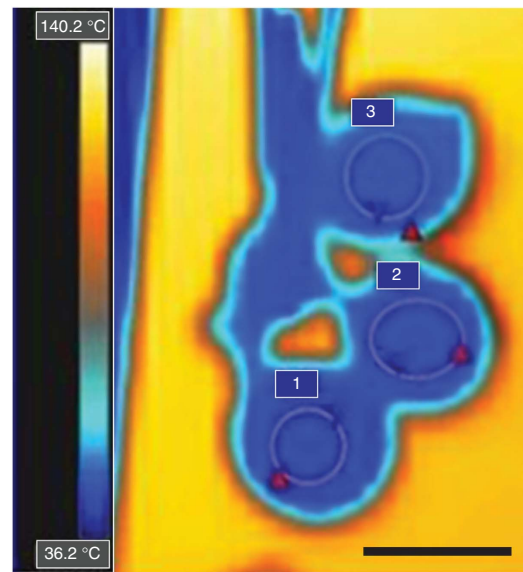


Figure 4 | Infrared thermogram of DM fruit. Three DM fruit in an infructescence being monitored by the FLIR E60 infrared camera, with numbers for each fruit indicating the area of interest where measurements were made. In this example, no one fruit has started to show thermogenesis, but the change will eventually occur in the fruit labelled '2', which will then become the test fruit; the fruit labelled '3' did not explode for another 5 min, so it was considered the control. Scale bar, 3 mm.

detect genuine thermogenesis. MDSC provides total heat flow data that are separated into reversing and non-reversing events. Both non-isothermal and isothermal experiments were performed. Non-isothermal MDSC has been used to measure thermogenic energy release (for example, from cytoplasmic male sterile rice mitochondria)¹⁴, and isothermal MDSC has been similarly used to monitor non-reversible heat flows and exothermic events (for example, exotherms associated with polymer curing)¹⁵; thus, both types of MDSC will readily identify thermogenesis. For the non-isothermal experiments, samples were equilibrated at 5°C and held under isothermal conditions for 10 min. The temperature was then ramped at $1.0^\circ\text{C min}^{-1}$ up to $65\text{--}80^\circ\text{C}$ with modulation $\pm 0.159^\circ\text{C min}^{-1}$. For the isothermal experiments, the samples were equilibrated at 25, 37 or 50°C . The temperature was modulated $\pm 0.159^\circ\text{C min}^{-1}$. The system was held under these conditions for 30–300 min. All data were analysed and integrated consistently using a sigmoidal baseline calculation¹⁶ to reveal trends and thermal events (TA Universal Analysis program, version 2000).

References

- Hawksworth, F. G. & Wiens, D. *Dwarf Mistletoes: Biology, Pathology, and Systematics* 709, 410 (U.S. Department of Agriculture, Forest Service, Agriculture Handbook, 1996).
- Unger, L. S. *Dwarf Mistletoes* (Forest Insect and Disease Survey, 1992).
- Hinds, T., Hawksworth, F. & McGinnies, W. Seed discharge in *Arceuthobium*: a photographic study. *Science* **140**, 1236–1238 (1963).
- Ross, C. M. Viscin cells in the dwarf mistletoe *Arceuthobium americanum* – “green springs” with potential roles in explosive seed discharge and seed adhesion. *Davidsonia* **17**, 75–86 (2006).
- Skotheim, J. M. & Madhevan, L. Physical limits and design principles for plant and fungal movements. *Science* **308**, 1308–1310 (2005).
- Ridley, H. N. *The Dispersal of Plants Throughout the World* (Ashford, 1930).
- Swaine, M. D. & Beer, T. Explosive seed dispersal in *Hura crepitans* L. (Euphorbiaceae). *New Phytol.* **78**, 695–708 (1977).
- Skubat, H., Nelson, T., Dong, A., Meeuse, B. & Bendich, A. Infrared thermography of *Arum* lily inflorescences. *Planta* **182**, 432–436 (1990).
- Kastberger, G. & Stachl, R. Infrared imaging technology and biological applications. *Behav. Res. Methods Instrum. Comput.* **35**, 429–436 (2003).
- Wagner, A., Krab, K., Wagner, M. & Moore, A. Regulation of thermogenesis in flowering Araceae: the role of the alternative oxidases. *Biochim. Biophys. Acta* **1777**, 993–1000 (2008).
- Moore, A., Bonner, W. & Rich, P. Determination of proton motor force during cyanide-insensitive respiration in plant-mitochondria. *Arch. Biochem. Biophys.* **186**, 298–306 (1998).
- Ross Friedman, C., Ross, B. & Martens, G. An antibody against a conserved C-terminal consensus motif from plant alternative oxidase (AOX) isoforms 1

- and 2 labels plastids in the explosive dwarf mistletoe (*Arceuthobium americanum*, Santalaceae) fruit exocarp. *Protoplasma* **250**, 317–323 (2013).
13. Kuraishi, S. & Nito, N. The maximum leaf surface temperatures of the higher plants observed in the inland sea area. *Bot. Mag. Tokyo* **93**, 209–220 (1980).
 14. Zhou, P.-J., Zhou, H.-T., Liu, Y., Qu, S.-S. & Zhu, Y.-G. Calorimetric and DSC studies of mitochondria isolated from cytoplasmic male sterile line of rice. *J. Therm. Anal. Calorim.* **76**, 1003–1013 (2004).
 15. Thomas, L. & Aubuchon, S. Heat capacity measurements using quasi-isothermal MDSC. *TA Instrum. Tech. Bull.* **230**, 1–5 (1999).
 16. Gabbott, P. A *Practical Introduction To Differential Scanning Calorimetry, In Principles And Applications Of Thermal Analysis* (ed. Gabbott, P. A.) 1–50 (Blackwell Publishing Ltd, 2008).
 17. Kelly, SK, Ross Friedman, C & Smith, RG Vesicular cells of the lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) fruit: development, cytochemistry, and lipid analysis. *Botany* **87**, 1177–1185 license 3530930744801.

Acknowledgements

Funding was provided by a Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grant to C.R.F. (no. 327214-2012) and an NSERC Undergraduate Student Research Award to R.A.J.d. (no. 448980-2013). We thank Dr R. Smith for his creative insights on DM. We also thank Dr N.J. Flood for her helpful comments on the manuscript.

Author contributions

C.R.F. was the principal investigator who conceptualized the research question and supervised the overall research. M.P. proposed both experiments. R.A.J.d. and C.R.F. performed all sampling of DM from the field. R.A.J.d., J.S.C. and M.P. designed and performed the IRT experiments, while K.A.R. and D.V.G. designed and performed the DSC trials. R.A.J.d. compiled the results and wrote the first draft of the manuscript, with M.P., K.A.R. and C.R.F. all contributing to the data analysis and writing.

Additional information

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions/>

How to cite this article: deBruyn, R. A. J. *et al.* Thermogenesis-triggered seed dispersal in dwarf mistletoe. *Nat. Commun.* 6:6262 doi: 10.1038/ncomms7262 (2015).



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

Author Correction: Thermogenesis-triggered seed dispersal in dwarf mistletoe

Rolena A.J. deBruyn, Mark Paetkau, Kelly A. Ross, David V. Godfrey, John S. Church & Cynthia Ross Friedman

Nature Communications 6:6262 doi: 10.1038/ncomms7262 (2015); Published 9 Feb 2015, Updated 25 Jun 2018

John S. Church, who provided access to the FLIR E60 infrared camera and provided guidance in its use, was inadvertently omitted from the author list in the originally published version of this Article. This has now been corrected in both the PDF and HTML versions of the Article.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

© The Author(s) 2018