Host manipulation by a herbivore optimizes its feeding behaviour

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Plant-manipulating herbivores can modify their host nutrients and defensive compounds to their own advantage. This generally results in enhanced performance of herbivores on modified tissues, but the anatomical and biochemical modifications undergone by host tissues could also modify the herbivores' behaviour. *Phloeomyzus passerinii* induces a reaction tissue in the bark of its susceptible poplar hosts, suggesting a host–plant manipulation by the aphid. We investigated whether the formation of this reaction tissue, confirmed by histological sections, affected the performance and feeding behaviour of the aphid. In addition we studied how host resistance affected these parameters by comparing two host genotypes of different susceptibility. Aphids developed faster on the preinfested susceptible host genotype, whereas no effect was detected on the resistant one, confirming an improvement in the host's nutritional value due to the preinfestation, in agreement with the nutrition hypothesis. The DC-EPG technique indicated that in both host genotypes, preinfestation shortened the delay until the first probe and increased both the rate and duration of sustained ingestion, and consequently host acceptance. This suggests that host manipulation can adjust not only the nutritional value of plant tissues at an optimal level for development but also host acceptance by the herbivores. In the resistant host genotype though, the number of probes and the duration of parenchyma exploration increased in the galled tissue, which was congruent with extensive cell wall modifications indicated by more extended induced lignification. The adaptive consequences of this modified feeding behaviour are discussed.

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The mismatch in nutrient contents between plant and animal tissues is one of the major hurdles preventing animals from efficiently exploiting plant resources (Schoonhoven, Van Loon, & Dicke, 2005). To cope with this nutritional imbalance, herbivores have developed diverse behavioural, anatomical and physiological adaptations. For instance, nutrient requirements can be fulfilled by compensatory feeding, i.e. eating more when nutrient concentrations are low, or dietary mixing, i.e. feeding upon complementary food sources (Bernays, 1998; Cook, Bailey, McCrohan, Nash, & Woodhouse, 2000; Fink & Von Elert, 2006; Simpson, Sibly, Lee, Behmer, & Raubenheimer, 2004). Modified digestive tracts or metabolism can also enhance nutrient assimilation, and symbioses with microorganisms allow the synthesis of missing essential nutrients that cannot be synthesized by herbivores (Bernays, 1998;

Douglas, 2013). However, some herbivores have developed an alternative, but nonexclusive, strategy consisting of a remodelling of both physical and biochemical components of plant tissues, leading to an improved nutritional value. Such host manipulations are encountered, for instance, in some leaf-mining insects (Body, Kaiser, Dubreuil, Casas, & Giron, 2013), sap-feeders (Sandström, Telang, & Moran, 2000) and gall-inducing animals, including insects, mites and nematodes (Abad, Favery, Rosso, & Castagnone-Sereno, 2003; Price, Fernandes, & Waring, 1987). These herbivores can turn the feeding sites in their host plant into mobilizing sinks that may accumulate nutrients such as amino acids (Body et al., 2013; Hammes et al., 2005; Koyama, Yao, & Akimoto, 2004; Matsukura, Matsumura, & Tokuda, 2012; Saltzmann, Giovanini, Zheng, & Williams, 2008; Sandström et al., 2000; Suzuki, Fukushi, & Akimoto, 2009), sugars or starch (Matsukura et al., 2012; Shannon & Brewer, 1980) and chemical elements (Brewer, Bishop, & Skuhravý, 1987), while the concentrations of defensive compounds can decrease (Nyman & Julkunen-Tiitto, 2000).

The nutrition hypothesis states that host manipulation adjusts the nutritional value of plant tissues to an optimal level for the

fitness of gall-inducers, which is probably also true for plant-manipulating herbivores in general (Hartley & Lawton, 1992; Price et al., 1987). This is supported by transplant experiments in which gall-inducing insects, and insects from other feeding guilds, exhibited faster larval growth (Diamond, Blair, & Abrahamson, 2008; Forrest, 1971; Koyama et al., 2004; Matsukura et al., 2012) and/or higher fecundity (Llewellyn & Hargreaves, 1986; Koyama et al., 2004) on galled than control tissues.

Besides physiological benefits, host manipulation may also affect different aspects of the feeding behaviour of the herbivores or their offspring. Since plant nutrients and secondary metabolites play a prominent role in host selection by herbivores (Schoonhoven et al., 2005), an improvement in the feeding substrate could enhance and quicken host acceptance. In addition, the anatomical and physiological changes that can occur in plant tissues during host manipulation (including gall differentiation), such as cell hypertrophy (increase in size), tissue hyperplasia (increase in cell multiplication) and cell wall modifications, may also change the probing behaviour, host acceptance and tissue consumption by the herbivores. For instance, short-duration preinfestations (i.e. previous probing and feeding) with nonmanipulating insect herbivores can modulate their feeding behaviour, by triggering induced resistance or susceptibility in their host plant (Dugravot et al., 2007; Gonzáles, Ramirez, Olea, & Niemeyer, 2002; Prado & Tjallingii, 1997; Sauge, Lacroze, Poëssel, Pascal, & Kervella, 2006). However, the effect of host manipulation on the way plant-manipulating herbivores in general feed upon their host has never been explored. Testing this hypothesis would require a model for which feeding behaviour is well known. Moreover, to allow easy comparisons between modified and control plant tissues, the model should preferentially initiate a reaction tissue that does not enclose it.

The woolly poplar aphid, *Phloeomyzus passerinii* (Aphididae: Phloeomyzinae), lives in bark crevices of poplar trunks (Blackman & Eastop, 1994). Detailed histological and behavioural investigations have demonstrated that it feeds upon the shallow tissues of the cortical parenchyma (tissue located in the bark between the cork and the phloem) (Pointeau et al., 2012). Aphids use an unusual intracellular and extracellular stylet pathway which, in susceptible hosts, ends with sustained intracellular ingestion in parenchyma cells (Pointeau et al., 2012). For several weeks after probing, while the aphids are settled on the bark and reproducing, the parenchyma undergoes anatomical and biochemical modifications resulting in a conspicuous bark swelling on stem cuttings (Dardeau et al., 2014). The modified parenchyma is characterized by thin-walled, hypertrophied cells, deprived of vacuolar phenolic compounds, and it has been hypothesized that *P. passerinii* induces a pseudogall (i.e. a simple gall inducing misshaping of a tissue) within the cortical tissues of its host (Dardeau et al., 2014). The aphid induces similar reaction tissues in poplar genotypes that are either susceptible or partially resistant, that is, in which resistance affects both the development and the feeding behaviour of *P. passerinii* (Pointeau et al., 2011, 2012). Yet in the resistant genotype, the reaction zone appears disorganized and cell wall lignification is diffuse and extended, whereas it is restricted to the stylet's insertion zone and to the periphery of the reaction tissue in the susceptible genotype (Dardeau et al., 2014).

In agreement with the nutrition hypothesis, if *P. passerinii* induces a pseudogall, we predicted that its development should be enhanced on the modified tissues of its host. In addition, the cellular transformations (hypertrophy, hyperplasia, cell wall modifications, etc.), along with the potential biochemical changes in cell contents, may modify the feeding behaviour of the aphid. Moreover, any factor that would modify or interfere with pseudogall formation, such as host resistance, should also consequently affect the performance and behaviour of the aphids. To test these three predictions we compared aphids' development and stylet penetration behaviour on normal and manipulated tissues of both a susceptible and a resistant poplar genotype.

METHODS

Two experiments were carried out, in 2010 and 2011, respectively, with the same aphid clone and the same plant material. In the first experiment, aphid performance parameters were compared between preinfested and control stem cuttings. After preinfestation by aphid colonies, fresh histological sections were prepared to observe plant reaction tissues. In the second experiment, the penetration of the aphid stylet was studied on preinfested stem cuttings by using the direct current electrical penetration graph (DC-EPG) technique. This technique is commonly used to investigate the hidden probing and feeding behaviour of insects with piercing–sucking mouthparts in plant tissues, by including an insect and its host plant in an electrical circuit (Giordanengo, 2014; Tjallingii, 1978). The electrical waveforms recorded can be related to the activity and stylet tip position within plant tissues. Both susceptible and resistant genotypes were used in each of these experiments.

Aphid Clone and Plant Genotypes

A monoclonal laboratory colony of *P. passerinii* was established from a parthenogenetic apterous (wingless) female, collected in October 2008 in Reboursin (France) on a *Populus × canadensis* Dorskamp genotype. Monoclonal colonies are routinely used for physiological and behavioural investigations with aphids since they ensure good experimental reproducibility. Comparisons of aphid performance parameters and host susceptibility between different monoclonal colonies of *P. passerinii* did not reveal marked differences. In addition, data from cytochrome oxidase I and II and cytochrome b genetic fragments analyses confirmed that the colony used for the study belonged to the most common haplotype in France (Pointeau, n.d.). The colony was maintained in the laboratory on stem cuttings (25 cm long) of the *P. × canadensis*. I-214 genotype under 21 ± 1 °C, 70 ± 10% relative humidity and a 16:8 h light:dark photoperiod.

Stem cuttings of two *P. × canadensis* genotypes commonly planted in France, I-214 and I-45/51, were used for the experiments. Previous investigations have confirmed that I-214 is highly susceptible to *P. passerinii*, whereas I-45/51 slows nymphal development and reduces the aphid's fecundity (Pointeau et al., 2011; Pointeau, Ameline, Sallé, Bankhead-Dronnet, & Lieutier, 2013). Stem cuttings (ca. 25 cm long, 2 cm diameter) were provided by the experimental nursery of Guéméné-Penfao (France). They were collected in the autumn of 2010 and 2011 for the two experiments, respectively, and kept at 2 °C in dry conditions until use. In June 2011 and June 2012, respectively, they were removed from storage, trimmed to 20 cm, and all buds except the terminal one were removed. The basal part was immersed in water for 5 days and then in liquid Exuberone for 20 min. The stem cuttings were planted in sand for 3 weeks and then transplanted into 2-litre pots containing a mix of 25% sand and 75% compost (Klasmann substrate 4 no. 267). Two weeks after planting, the rooted stem cuttings were transferred to a growth chamber with controlled environment until the end of the experiment (21 ± 1 °C, 70 ± 10% relative humidity, 16:8 h light:dark photoperiod and a photon flux density of 300 μmol/m^2/s). We used 55 stem cuttings of each host genotype for the first experiment and 50 for the second.