Journal of Chemical Ecology (2020) 46:84–98
https://doi.org/10.1007/s10886-019-01130-z

REVIEW ARTICLE

Phytochemicals Involved in Plant Resistance to Leporids and Cervids: a Systematic Review

Emilie Champagne[1,2] · Alejandro A. Royo[3] · Jean-Pierre Tremblay[1,4] · Patricia Raymond[2]

Received: 4 September 2019 / Revised: 4 September 2019 / Accepted: 2 December 2019 / Published online: 20 December 2019

Abstract
Non-nutritive phytochemicals (secondary metabolites and fibre) can influence plant resistance to herbivores and have ecological impacts on animal and plant population dynamics. A major hindrance to the ecological study of these phytochemicals is the uncertainty in the compounds one should measure, especially when limited by cost and expertise. With the underlying goal of identifying proxies of plant resistance to herbivores, we performed a systematic review of the effects of non-nutritive phytochemicals on consumption by leporids (rabbits and hares) and cervids (deer family). We identified 133 out of 1790 articles that fit our selection criteria (leporids = 33, cervids = 97, both herbivore types = 3). These articles cover 18 species of herbivores, on four continents. The most prevalent group of phytochemicals in the selected articles was phenolics, followed by terpenes for leporids and by fibre for cervids. In general, the results were variable but phenolic concentration seems linked with high resistance to both types of herbivores. Terpene concentration is also linked to high plant resistance; this relationship seems driven by total terpene content for cervids and specific terpenes for leporids. Tannins and fibre did not have a consistent positive effect on plant resistance. Because of the high variability in results reported and the synergistic effects of phytochemicals, we propose that the choice of chemical analyses must be tightly tailored to research objectives. While researchers pursuing ecological or evolutionary objectives should consider multiple specific analyses, researchers in applied studies could focus on a fewer number of specific analyses. An improved consideration of plant defence, based on meaningful chemical analyses, could improve studies of plant resistance and allow us to predict novel or changing plant-herbivore interactions.

Keywords Secondary metabolites · Deer · Hare · Diet selection · Defense metabolite · Secondary compound

Introduction

Plants possess a large array of traits providing resistance to herbivores, either promoting tolerance (i.e. regrowth) or the physical and chemical avoidance of herbivory (Hester et al. 2006). Chemical avoidance relies on anti-nutritive phytochemicals, which reduce consumption because of their toxicity or their negative impacts on digestion (Iason 2005) and, ultimately, their negative impacts on animal fitness (DeGabriel et al. 2009; Iason 2005). These phytochemicals include plant secondary metabolites (PSM; Fraenkel 1959; Raguso et al. 2015) and indigestible structural components such as fibre (Laca and Demment 1996). Although anti-nutritive phytochemicals are not always the main factor determining diet composition (Agrawal and Weber 2015; Carmona et al. 2011), they are a component of the co-evolutionary arms race between plants and herbivores (Feeny 1991; Moore et al. 2014) and can structure plant-herbivore dynamics, for example by their effects on herbivore population dynamics and spatial distribution (Anderson et al. 2015; Moore et al. 2010; Underwood et al. 2005).

A major hindrance to the study of anti-nutritive phytochemicals is that we still lack consensus as to which chemical compounds determine plant resistance, especially with mammalian herbivores (DeGabriel et al. 2014). Multiple PSM have no effect against herbivores or are simply inactive, but are

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10886-019-01130-z) contains supplementary material, which is available to authorized users.

✉ Emilie Champagne
Emilie.Champagne@mffp.gouv.qc.ca

1 Département de biologie & Centre d'étude de la forêt, Université Laval, QC, Québec, Canada

2 Direction de la recherche forestière, Ministère des Forêts, de la Faune et des Parcs, QC, Québec, Canada

3 USDA Forest Service Northern Research Station, 335 National Forge Road, Irvine, PA 16365, USA

4 Centre d'études nordiques, Université Laval, QC, Québec, Canada

retained as potential precursors for new active compounds (screening hypothesis; Jones et al. 1991; Moore et al. 2014). Moreover, anti-nutritive phytochemicals are often lumped into simplified groups (e.g., total phenolics, crude fibre) within which only a handful of compounds may have an anti-herbivory effect (Tahvanainen et al. 1985). As a result, the effects of phytochemicals on herbivore food choices are often contradictory, even for a specific herbivore species (Felton et al. 2018). Tannins, for example, are a widely studied group of PSM because they can negatively affect ruminant digestion (McSweeney et al. 2001; Robbins et al. 1987a; Robbins et al. 1987b), and should thus be avoided by ruminants. Their effects on plant resistance to herbivory, however, are variable. For a ruminant like *Odocoileus virginianus*, there is evidence of positive (Adams et al. 2013), negative (Chapman et al. 2010), or neutral (Holeski et al. 2016) effects of tannin content on consumption, for three different forage types (respectively: *Juniperus ashei*, pelleted diet and *Populus tremuloides*). This variability is not surprising as complete avoidance of non-nutritive phytochemicals is impossible and herbivores have developed strategies to tolerate them (Iason and Villalba 2006). Nevertheless, our understanding of the effect of these phytochemicals on plant resistance could be improved by studying specific active compounds (Felton et al. 2018).

Recently, ecologists have advocated that complete characterizations of chemical content may advance our understanding of mammalian herbivore food choices (Felton et al. 2018; Wam et al. 2017). These characterizations should include several nutritional drivers such as energy, proteins, minerals, nutrients, fibre and PSM (Felton et al. 2018). Detailed nutritional analyses, however, are not always possible because of the costs and expertise required. Moreover, this level of specificity may be unnecessary for many objectives. For conservation or management, we might want to assess the potential resistance of an introduced plant species to herbivores (e.g. Averill et al. 2016). In these contexts, analyses of the composition and abundance of select phytochemicals, known to influence plant resistance, may suffice and might even provide a more parsimonious explanation to plant resistance.

The objective of this study was to review the effects of non-nutritive phytochemicals (PSM and fibre) on plant resistance to leporids and cervids, with the underlying goal of identifying appropriate phytochemical proxies of plant resistance to herbivores. We focused on leporids and cervids, because of their potential to influence forest regeneration (Côté et al. 2004; Oldemeyer 1983) and the rich literature amenable to review concerning them. Moreover, their fundamentally different digestive systems could allow us to identify patterns and trends in phytochemicals effects. Leporids are hindgut fermenters, and fermentation occurs in the caecum after phytochemical digestion and absorption, while cervids are foregut fermenters, and fermentation occurs in the rumen before chemical digestion. These differences may affect the influence of phytochemicals on digestion. For example, undigested tannins can negatively impact the metabolism of the rumen microorganisms (McSweeney et al. 2001), and thus may have stronger effects on foregut fermenters than on hindgut fermenters. Leporids and cervids also have fundamentally different approaches to fibre digestion: hindgut fermenters excrete rapidly large, poorly digestible particles (Hirakawa 2001) to constantly provide their digestive system with high-quality food (Penry and Jumars 1987). Foregut fermenters retain poorly-digestible particles for long periods and ruminate those particles, thereby digesting a higher proportion of the digestible fibre than hindgut fermenters (Steuer et al. 2013). Consequently, fibre concentration could be more critical for leporids than for cervids. Additionally, patterns and trends in the effects of phytochemicals on resistance could be linked to differences in study conditions, such as the experimental design or the use of captive animals. To minimize bias in our review, we performed a systematic literature search and used a detailed protocol to find, evaluate and summarize research articles. By identifying the phytochemicals and study conditions affecting plant resistance, we hope to highlight areas of research that need investigation and provide researchers and managers with guidance on the selection of chemical analyses when a complete chemical analysis is not possible or desirable.

Methods and Materials

Literature Search We aimed to find articles investigating the relationship (correlations and causations) between non-nutritive phytochemicals and plant resistance to cervids and leporids. We defined non-nutritive phytochemicals (hereafter phytochemicals) as all compounds classically defined as PSM (e.g., phenolics and terpenes) but we also included fibre as fibre can act as anti-nutritive constituents (Laca et al. 2001). Selected articles needed to describe how consumption by herbivores varied among plants with different phytochemical concentrations, to determine how the phytochemical content was linked to plant resistance. We determined keywords addressing three topics: 1) food choices; 2) herbivore type; 3) chemistry (see Online Resource 1 for the list of keywords). We conducted the search on Web of Science Core Collection on 17 January 2018 and use wildcards (*) to account for various word spellings. We directly identified 1637 articles and added 126 articles through the references section of the retrieved articles (see Online Resource 1). We added 27 additional records from previous knowledge and from a recently published systematic review of the nutritional drivers of food selection (Felton et al. 2018). We chose to proceed directly to the full-text assessment of these additional records instead of going through the screening phase (see article selection below).

Article Selection and Data Extraction We had two criteria for a priori article rejection: 1) the article was not about a cervid or a leporid; 2) the article did not report relationships between a varying phytochemical content and consumption. While determining the suitability of articles, we refined selection by using three rejection criteria a posteriori: 1) the article used second-hand phytochemical data not obtained by the article's authors; 2) the article concerned habitat selection rather than consumption; 3) the article was a review paper. To determine if the article could be rejected based on all the criteria listed above, we first read the title and abstract of each article (see Online Resource 1 for the number of articles retained at each step and the list of selected articles). For articles that passed this screening phase, we consulted the full text and classified them between 'excluded' and 'included' (eligibility phase).

A single observer (E. Champagne) reviewed all articles and filled a consultation data sheet for each article included in the systematic review. The consultation data sheets included five categorical variables: 1) Herbivore type (cervid, leporid, both); 2) Plant type (coniferous, broad-leaved, forbs, ferns, grass, lichens, artificial food); 3) Phytochemical groups studied (nitrogen-based compounds, phenolics, terpenes and fibre). We also noted whether the effects of nitrogen or protein were tested; 4) Phytochemical comparison type (varying phytochemical concentration among different plant species or within a plant species); 5) Study type (captive trial, field trial, field observations). Captive and field trials could take the form of cafeteria-style experiments (i.e. free-choice feeding from several forage type, offered simultaneously), or no-choice experiments (i.e. comparison of intake for different forages, offered sequentially). In field observations, the chemical content of browsed plants was often compared to the content of unbrowsed plants. For cervids, we included a sixth categorical variable, i.e. herbivore feeding type (concentrate selector, intermediate feeder, roughage eater), based on Hofmann (1989) and Loison et al. (1999) (see Table 1 for exceptions). We also summarized articles' main results concerning the relationship between phytochemical content and consumption, as interpreted by the authors (see Online Resource 2 for the consultation data sheet). This summary included, but was not restricted to, the phytochemicals correlated and not correlated to consumption, the presence of thresholds in consumption-phytochemical relationships, the interaction with other phytochemicals or nutritional constituents. We verified current taxonomic information, including common names, of animals with the package *taxize* in R (Chamberlain et al. 2018), using the Integrated Taxonomic Information System (ITIS; http://www.itis.gov).

We used contingency tests to evaluate the effect of investigatory approaches variables (phytochemical comparison type, study type) on the probability of finding a relationship (positive or negative) between consumption and phytochemical concentration (main phytochemical groups). We also used contingency tests for phytochemical subgroups with a large number of articles. We used a χ^2 contingency test when its assumption was met (less than 20% of the contingency table's cells had a frequency lower than 5). When the assumption was not met, we used a Fisher's exact test for test involving comparison type. For study type, we did not perform a statistical test when the assumption was not met, as the Fisher's exact test is designed only for 2×2 contingency tables. This approach should be interpreted with caution, as vote-counting is not an indicator of the actual strength of the relationship between consumption and phytochemical concentration, but rather a description of the available dataset. Moreover, reported relationships in several selected articles were not subjected to statistical tests. In these cases, our classification of relationship vs no relationship classes is based on our interpretation of the article's content. We evaluated the possibility of using meta-analytical estimates, but were prevented from doing so by the diversity of study design and result formatting.

Results

Description of the Selected Articles Following the identification, screening and eligibility phases, we identified 133 out of 1790 articles that fit our selection criteria (Online Resource 1 for bibliographic references and Online Resource 2 for summary tables of consultation data sheets). Selected articles will be cited throughout this article using their identification number between brackets to differentiate them from articles that do not belong to the systematic review.

Of the 133 articles, 33 studied leporids, 97 studied cervids and three studied both (Table 1). The species distribution reflects the geographical distribution of the studies (Table 1, Fig. 1). Most articles included woody plants, both for leporids (coniferous = 9, broad-leaved = 25, $n = 3$ for both forbs and grasses) and cervids (coniferous = 50, broad-leaved = 45, forbs = 10, grasses = 9). Thirteen articles for leporids and 19 articles for cervids used artificial food, that is, pellets or plants with added phytochemicals.

Study type frequency differed between the two herbivore types: leporid articles used captive trials more often than other study types, while captive trials and field observations were similarly represented in cervid articles (Fig. 2). For both herbivore taxa, the majority of articles tested the effects of variation in phytochemical concentration among plants of the same species (hereafter, within-species comparison) rather than among plants of different species (hereafter, among-species comparison; Fig. 2).

The selected articles mostly investigated phytochemical in three groups: phenolics, terpenes and fibre (Table 2). Twelve

Table 1 Number of selected articles per herbivores species in a systematic review of the effect of plant secondary metabolites on forage consumption by leporids and cervids

Latin name	Common name	Number of articles	Feeding type
Leporids			
Brachylagus idahoensis	Pygmy rabbit	6	
Lepus americanus	Snowshoe hare	18	
Lepus timidus	Mountain hare	7	
Lepus europaeus	European hare, brown hare	2	
Oryctolagus cuniculus	European rabbit	2	
Sylvilagus floridanus	Eastern cottontail	2	
Sylvilagus nuttalli	Mountain cottontail, Nuttall's cottontail	2	
Cervids			
Alces alces	Moose, Eurasian elk	22	Concentrate selector
Capreolus capreolus	European roe deer, Western roe deer	5	Concentrate selector
Cervus elaphus[a]	Red deer, Wapiti, elk	22	Intermediate feeder
Cervus nippon	Sika deer	4	Intermediate feeder
Rusa timorensis	Javan rusa, Timor deer	1	Intermediate feeder[b]
Rusa unicolor	Sambar	1	Intermediate feeder[c]
Dama dama	Fallow deer	12	Intermediate feeder
Moschus berezovskii	Forest musk deer, Chinese forest musk deer	1	Intermediate feeder[d]
Odocoileus hemionus[a]	Mule deer	22	Concentrate selector
Odocoileus virginianus	White-tailed deer	18	Concentrate selector
Rangifer tarandus	Reindeer[e]	2	Intermediate feeder

Subspecies were grouped and 12 articles (leporids, $n = 3$; cervids, $n = 9$) included more than one species. For cervids, feeding types are based on Hofmann (1989) and Loison et al. (1999): 1) concentrate selectors (CS or browsers) consume plants with high cell contents and feed frequently, mostly woody plants; 2) roughage eaters (RE or grazers) consume mostly grass; 3) intermediate feeders are intermediates between CS and RE

[a] Including subspecies

[b] Based on de Garine-Wichatitsky et al. (2003)

[c] Classed as roughage eater by Loison et al. (1999), but the article concerned demonstrate that it is an intermediate feeder (Semiadi et al. 1995)

[d] Based on genus information available in Loison et al. (1999)

[f] All articles concerning *Rangifer tarandus* are in Europe, and thus not refer to caribou

articles also reported measure of resin content, which is not a chemical class per se, but is rich in terpenes and phenolics (Table 2, Fig. 3a). Two articles investigated alkaloids (nitrogen-based PSM), although one did not detect their presence [*69*], while the other found a negative relationship between alkaloid content and consumption [*116*]. Alkaloids are found in approximately 20% of angiosperms (Harborne 1991), and their absence from the selected articles could indicate a strong avoidance or the near-absence of alkaloids in the plants studied. We do not discuss alkaloids further in this systematic review.

For both leporids and cervids, phenolics were the most studied class of phytochemicals (Fig. 3a). We further subdivided the phenolics group into four subgroups based on the frequency of measures reported in the selected articles: total phenolics, tannins, phenolic glycosides and all other phenolics (Fig. 3b). Tannins were the most studied subgroups for cervids, while total phenolics are most studied subgroups for leporids (Fig. 3b). Terpenes were also divided in subgroups according to their number of isoprene units: monoterpenes (2 units), sesquiterpenes (3), diterpenes (4) and triterpenes (5). Monoterpene was the most studied subgroups of terpenes for both types of herbivores (Fig. 3c).

Phenolics and terpenes are rarely investigated in tandem (leporids = 6, cervids = 11). Thirty-nine articles investigated fibre and phenolics or terpenes (leporids = 8, cervids = 31) and ten articles (leporids = 3, cervids = 7) investigated phenolics, terpenes and fibre. None investigated all categories of compounds presented in Fig. 3a. More than half of the cervids and leporids' articles also measured the concentration in nitrogen, in crude proteins or, less frequently, in digestible proteins (Fig. 3a).

Approximately 17% of the selected articles did not find relationships (positive or negative) between phytochemical content and consumption (Tables 3, 4, 5). Some authors used qualitative analyses or discussed relationships that were not statistically significant [e.g. *67, 72, 76, 91, 110, 125*]. For leporids, only six articles out of 36 did not report statistically

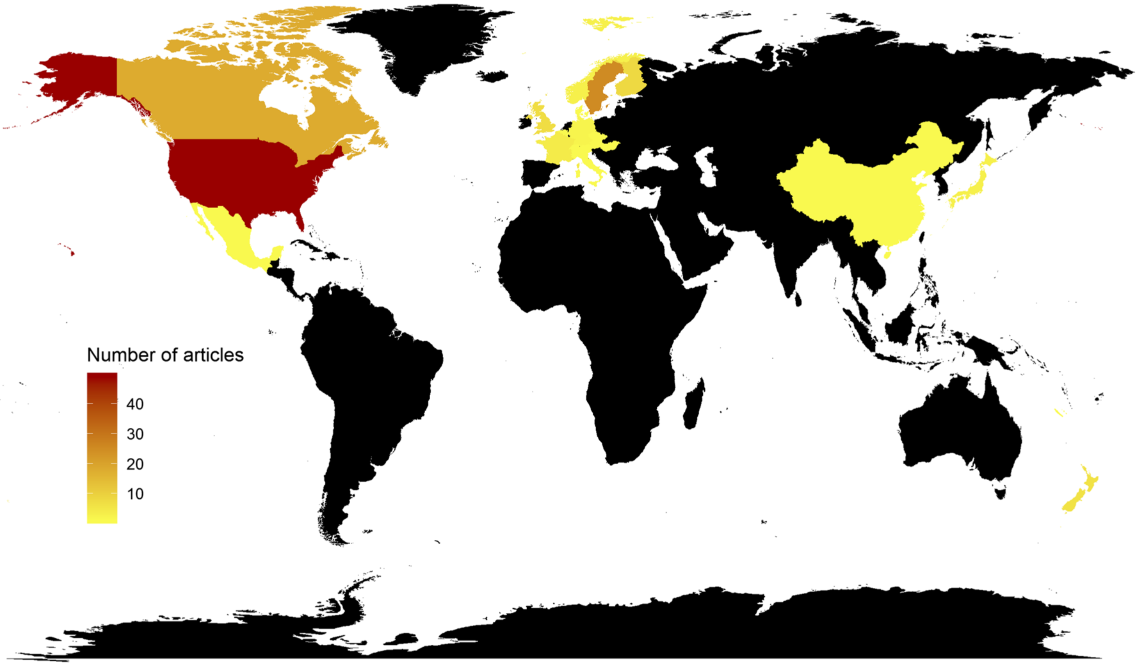

Fig. 1 Geographical distribution of the studies selected in a systematic review of the effect of non-nutritive phytochemicals on forage consumption by leporids and cervids

significant results for phytochemicals [*4, 8, 15, 23, 24* and *36*], but even those articles reported tendency or qualitative relationships, except [*23*]. Generally, the articles reported relationships with only a subset of the studied phytochemicals. The likelihood of finding a relationship is not linked to the investigatory approach used, except in two cases considering all phytochemicals (Table 3).

The following sections summarize the effects on consumption for each phytochemical groups described in Table 2 per herbivore type.

Fig. 2 Venn diagrams for type of study (Captive trials, Field trials and Field observations) and type of comparison (Among-species or Within-species) for both types of herbivores (total number of articles: leporids = 37, cervids = 100) in selected articles from a systematic review of the effect of non-nutritive phytochemicals on forage consumption by leporids and cervids

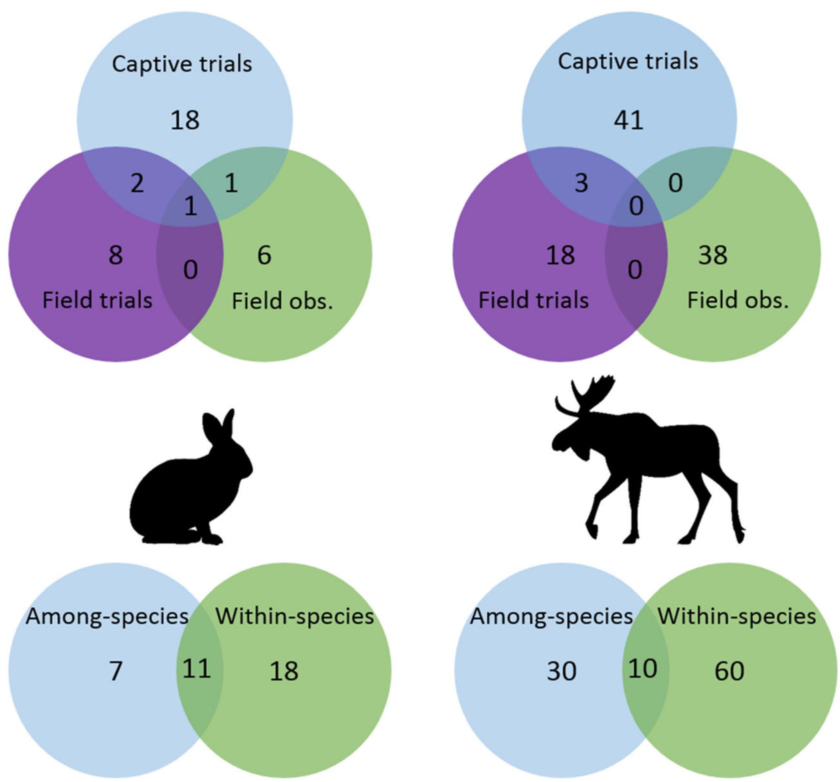

Table 2 Characteristics and effects on herbivores of groups and subgroups (in italics) of phytochemicals present in the systematic review of the effect of plant secondary metabolites on forage consumption by leporids and cervids

Phytochemical group	Characteristics	Effects on herbivores
Phenolics	• Compounds with an hydroxyl group bonded to an aromatic hydrocarbon group • 10,000 compounds synthesized from several metabolic pathways[a] • Wide distribution among flowering plants[b]	• The effect against herbivores is linked to specific subgroups of phenolics
Tannins	• Structural units with free phenolic groups, characterized by their ability to bond with proteins[c] • Can be divided into two types: Condensed (widespread)[b,d] Hydrolyzable (only in dicotyledons) [e] • Hydrolyzable tannins can be counteracted by microbial metabolism, but condensed tannins cannot[g]	• Reduce protein availability and fibre digestibility[f,g] • Toxic by binding with digestive enzymes and proteins[g] • Astringent[h] • Positive effects: reduced the influence of other PSM, increased protein denaturation (at low tannin concentration) and retention[e]
Phenolic glycosides	• Phenolics bounded to a sugar	• Toxic, following the hydrolysis after ingestion[b,i] • Major negative effects on reproduction and survival of domestic herbivores[j] • Effects against wildlife uncertain[k]
Flavonoids	• Polyphenols with a benzo-γ-pyrone structure[l] • Pigments • Ubiquituous in plants[l]	• Antioxidant and anti-microbial effects[l,m] • Do not appear to have significant toxic activity on mammals[b,l] • Catechins (subgroup) have a bitter taste, are astringent and have tannin-like activity[b]
Stilbenoids	• Typical of *Pinus* and *Eucalyptus*[n]	• Antifungal activity[n]
Phenolic acids	• In the selected studies: Chlorogenic acid (widespread among plants)[n] Shikimic acid	• Chlorogenic acid: Associated with defence against pathogens in plants[o]. Negative effect on invertebrates[p]
Terpenes	• Compounds with a common biosynthetic origin and with the same basic structure (isoprenoid)[q] • Widespread in vascular plants, especially common in conifers[q]	• Antibacterial and antifungal activity that can reduce digestion by hindgut and foregut fermenters[r,s,t]
Monoterpenes	• 2 isoprene units • Volatile and odorant	• Attraction or deterrence by smell[q]
Sesquiterpenes	• 3 isoprene units • Volatile	• Some are toxic to vertebrates (e.g. sesquiterpene lactones)[u,v] • Bitter taste[q]
Diterpenes	• 4 isoprene units	• Poisonous, irritants and carcinogenic[b]
Triterpenes	• 5 isoprene units	• Toxic to mammals (e.g., saponins, cardenolids), although toxicity is dubious in natural environment[q] • Bitter taste (e.g., cucurbitacins)[q]
Resin	• Ether-extractable fraction of woody plants (especially conifers) • Excreted in response to damage • Rich in PSM, such as terpenes and phenolics	
Fibre	• Primary metabolites, essential for plant structure, especially in woody plants • Several different compounds, mostly hemicellulose, cellulose and lignin	• Hemicellulose can be digested • Digestion of cellulose requires symbiotic microbiota • Lignin is indigestible[c] • Strong influence on digestible content of plants[f]

[a] Taiz and Zeiger (2002)
[b] Harborne (1991)
[c] Hagerman and Butler (1991)
[d] Ayres et al. (1997)
[e] Bernays et al. (1989)
[f] Robbins et al. (1987b)
[g] McSweeney et al. (2001)
[h] Mali and Borges (2003)
[i] McArthur et al. (1991)
[j] Majak (2001)
[k] Lindroth (2000)
[l] Kumar and Pandey (2013)
[m] Malhotra et al. (1996)
[n] Constabel (1999)
[o] Hammerschmidt and Smith-Becker (1999)
[p] Liu et al. (2017)
[q] Gershenzon and Croteau (1991)
[r] Gershenzon and Dudareva (2007)
[s] Nagy and Tengerdy (1968)
[t] Schwartz et al. (1980)
[u] Picman (1986)
[v] Picman et al. (1982)

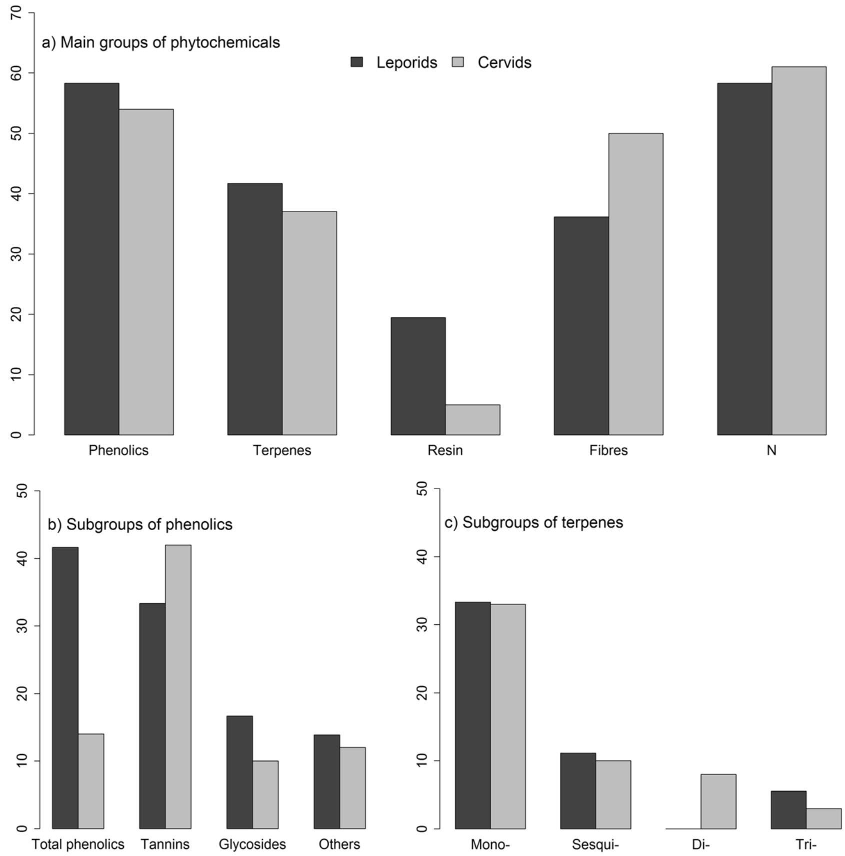

Fig. 3 Proportion of articles selected in a systematic review of the effect of non-nutritive phytochemicals on forage consumption by leporids and cervids in each a) chemical composition groups (total number of articles: leporids = 37, cervids = 101), in b) subgroups of phenolics (leporids = 21, cervids = 54) and c) subgroups of terpenes (leporids = 15, cervids = 37)

Phenolics

Total Phenolics

A small number of articles with total phenolic measure (Tables 4, and 5) reported a negative relationship with consumption [leporids: *11*, *14*, *20, 27, 35*; cervids: *41, 65, 74, 98*]. Two articles also demonstrated that phenolic composition can explain forage choices [*Alces alces*: *74, 75*].

Tannins

Leporids 50% of articles investigating tannins found relationships between tannins and consumption by leporids (Table 4). In [*1*], tannins were positively related to consumption, but nitrogen content explained diet choices. Conversely, negative relationships between consumption and different estimates of tannin content were reported [*27, 30, 33, 35* and *36*]. These relationships were not always consistent, for example, the least preferred plant species had the lowest protein-complexing phenolics content [30].

Cervids The significant relationships reported between consumption and tannins were inconsistent (Table 5), even when considering a single cervid species. For species with several articles testing tannins (*Alces alces*, *Odocoileus virginianus*, *Capreolus caprioles* and *Cervus elaphus)*, studies reported both positive [*37, 77, 79, 86, 118, 122, 123*] and negative food selection for tannins [*46, 51, 52, 71, 74, 81, 112, 122*]. In some articles, tannin avoidance seemed to be triggered by low protein availability in forage [see *37* and *71*]. More insights are gained with the study of *Dama dama*, which was the subject of eight articles with tannins. Although this species reduced food intake as concentrations of condensed tannins or hydrolyzable tannins increased, *D. dama* will consume high-tannin food even in the presence of a low-tannin option [*42–45, 47–49, 70*; all captive trials]. This behaviour suggests that tannin intake is regulated to a certain amount. Three other cervids also seemed to adjust their diet to achieve a moderate tannin intake (*Odocoileus virginianus* [*37, 51*], *Cervus capreolus* [*52, 79, 81*], and *Moschus berezovskii* [*85*]). Only one of the five field trial studies found relationships between consumption and tannin content which suggests tannins have little effects on cervids' consumption in natural environments.

Phenolic Glycosides

Leporids Negative relationship between consumption and phenolic glycosides concentrations were reported in half of the studies concerning these compounds (Table 4). Specific phenolic glycosides or derivatives were avoided [*21, 26*], whereas others were not [*35*]. For example, *Lepus timidus*

Table 3 Influence of the investigatory approach on the probability of finding a relationship between phytochemical content and herbivore consumption as evaluated by contingency tests, per herbivore type and per main groups of compounds

Phytochemical group	Leporids		Cervids	
	Comparison type	Study type	Comparison type	Study type
All groups	***P* = 0.025** No relationship A = 5, W = 1 Relationship found A = 13, W = 28	No relationship found CT = 2; FT = 2; FO = 1 Relationship found CT = 20; FT = 9; FO = 7	$\chi^2 = 3.14$ df = 1 *P* = 0.076	$\chi^2 = 7.03$ df = 2 ***P* = 0.030** No relationship found CT = 5; FT = 8; FO = 6 Relationship found CT = 39; FT = 13; FO = 32
Phenolics	*P* = 0.41	No relationship found CT = 2; FT = 2; FO = 2 Relationship found CT = 11; FT = 7; FO = 2	*P* = 0.24	No relationship found CT = 6; FT = 3; FO = 6 Relationship found CT = 22; FT = 5; FO = 13
Terpenes	P = 0.41	No relationship found CT = 1; FT = 0; FO = 1 Relationship found CT = 3; FT = 2; FO = 10	*P* = 0.65	No relationship found CT = 4; FT = 1; FO = 2 Relationship found CT = 13; FT = 8; FO = 11
Resin	*P* = 1.00	No relationship found CT = 0; FT = 1; FO = 0 Relationship found CT = 5; FT = 2; FO = 2	P = 1.00	No relationship found CT = 0; FT = 2; FO = 0 Relationship found CT = 2; FT = 1; FO = 1
Fibre	P = 1.00	No relationship found CT = 5; FT = 2; FO = 3 Relationship found CT = 2; FT = 2; FO = 3	$\chi^2 = 1.13$ df = 1 *P* = 0.29	$\chi^2 = 3.62$ df = 2 *P* = 0.16

When possible, we used a χ^2 contingency test. We used Fisher's exact test for test involving comparison type with low sample size. For study type, we did not perform a statistical test when the assumptions were not met and we report the number of studies in each category. A statistically significant *p* value for the contingency test ($\alpha = 0.05$) indicates that the probability of finding significant results differs between PSM comparison type (A = among species, W = within species) or among study types (CT = captive trials, FT = field trials or FO = field observation). Articles with both types of comparisons or with more than one study types were counted twice

avoided the phenolic glycoside fraction of *Salix* spp. bark extract [*32*], but did not avoid flavonol glycosides [*22*].

Cervids Negative relationships are reported for phenolic glycosides in the majority of studies considering them (Table 5), and especially for total phenolic glycosides [*64, 106*], fractions containing phenolic glycosides [*111*], and subgroups of glycosides [e.g. myricetin, salicylates, *73, 102, 112*]. Moreover, there was a negative effect on consumption of specific glycosides [*73, 74, 102, 106, 111*], such as salicortin [*74, 102, 106*] and tremulacin [*39, 102, 106*].

Other Phenolics

Flavonoids

A total of eight articles investigated flavonoids either with leporids or cervids, and six of these reported negative relationships with consumption (Tables 4, 5). For leporids, one study found a strong negative correlation (r = −0.72, $p < 0.05$; exact *p* value not available) between the concentration of flavonoid aglycones and plant consumption by *Lepus timidus* [*22*]. Catechins (a type of flavonoid, Table 2) were not related to consumption by *L. americanus* [*22*] but the addition of the catechin fraction of *Salix* spp. twigs reduced feeding by *L. timidus* [*32*].

Stilbenoids

Stilbenoids were studied only with leporids, and all studies (*n* = 3) demonstrated negative relationships between consumption and specific stilbenoids (e.g. pinosylvin; Table 4), although the effects of these phytochemicals were variable and differed in strength.

Phenolic Acids

Five studies reported positive relationship between two specific phenolic acids and consumption by cervids: chlorogenic acid (*Odocoileus hemionus* [*97, 98, 103*], *Alces alces* [*102*]) and shikimic acid (*A. alces* [*111*]). The phenolic acids studied with leporids were not linked to consumption (Table 4).

Table 4 Summary table of relationships among phytochemicals and consumption by leporids in selected articles

Phytochemical group	Positive relationships	Negative relationships	Specificities
Phenolics	1/21	14/21	
Total phenolics	1/15	5/15	
Tannins	1/12	5/12	All articles with woody plants. All but one with *Lepus* species.
Phenolic glycosides	0/6	3/6	All articles with *Lepus* species. Articles with total phenolic glycosides [15, 32], total flavonol glycosides [22] or specific compounds [21: 2,4,6-trihydroxydihydrochalcone 1, 22: five compounds, 26: Salicaldehyde and 6-Hydroxycyclohex-2-ene-one, 35: salicin, picein and triandrin].
Other phenolics			One article [22] included several specific phenolics and phenolics from various groups. Other compound studied: 2-phenetyl cinnamate [19].
Flavonoids	0/2	2/2	All studies with *Lepus* species. Flavonoids investigated: flavonol glycosides, flavonoid aglycones [22] and catechins [22, 32].
Stilbenoids	0/3	3/3	Stilbenoids investigated: pinosylvin and methyl ether derivates [18, 19, 30], pinostrobin [19], resorcinol [19].
Phenolic acids	0/1	0/1	Compounds studied: Caffeoyl quinic acids, cinnamic acids derivatives [22].
Terpenes	1/15	13/15	Articles mostly confined to woody plants (broad-leaved = 7 articles, coniferous = 4). All articles concerning *Brachylagus idahoensis* and/or *Sylvilagus nuttalli* included terpene analyses. None of the articles investigated total terpene content, although two articles measured groups that could be equivalent to total terpene content [3, 27].
Groups of terpenes	0/7	3/7	Negative relationships reported with essential oil yield [3], total triterpenes [22], steam distillate composed of sesquiterpenes [27].
Specific terpenes	1/13	12/13	Most of the terpenes with negative relationships are monoterpenes (1,8-cineol, artemiseole, α-thujone, camphor), at the exception of glaucolide-A [sesquiterpene lactone; 2], germacrone [sesquiterpene; 25] and papyriferic acid [triterpene; 27]. Positive relationship with bornyl acetate [34].
Resin	0/7	6/7	6 articles with *Lepus americanus*, 1 article with *Lepus timidus*.
Fibre	1/13	6/13	Fibre effects have been investigated mostly for woody plants (11 articles), with all leporids except *Sylvilagus floridanus*. One article found both positive and negative relationships, depending on the type of fibre: positive with cellulose and negative with lignin [7]. Although not significant, [4] also report a positive relationship with hemicellulose and negative with lignin.

Articles are classified in groups of chemicals and in positive and negative relationships, based on summary realized during the systematic review. Classification in positive and negative relationship is a simplification of the results (see Online Resource 2 for a detailed summary of each article)

Terpenes

Leporids Plant consumption by leporids seemed related to specific monoterpenes, sesquiterpenes and/or triterpenes rather than to terpenes subgroups (Table 4). For example, the monoterpene 1,8-cineole, also known as cineol or eucalyptol, was related to lower consumption in four studies [*5*, *16*, *26*, *28*]. One article reported a positive relationship between a monoterpene (α-pinene) and consumption [*3*].

Cervids Relationships between consumption and terpenes reported were generally negative (Table 5) and these negative relationships seemed related to total terpene content or to terpene composition [37, 83]. Articles that included terpene groups and specific terpenes either found an effect of both [*37, 50, 64, 90, 92, 96, 111*], or no effect at all [*67, 87, 97, 105, 110*]. In three articles, there was a significant relationship between terpene groups and consumption but not between specific compounds within these groups and consumption [*56, 57, 99*]. In only three studies, plant resistance was related to specific monoterpenes but not to terpene groups [*27, 114, 87*]. A few cases of positive relationships are also reported. One positive relationship can be explained either by a positive food selection for protein [*132*]. The other positive relationships reported are inconsistent: diterpenes are positively correlated to preference by *Capreolus capreolus* [*79*] and browsing by *Odocoileus hemionus* [*83*]. Browsing by *O. hemionus*, however, was negatively related to diterpenes in another article with the same plant species [*84*]. Diterpenes and especially cyclic diterpenes were also negatively linked to browsing by *Alces alces*, but only in dry forest sites, suggesting an effect of site quality on diterpene profile [*92*].

Resin

Leporids Six articles reported a negative relationship between resin concentration and consumption (Table 4) on multiple

Table 5 Summary table of relationships among phytochemicals and consumption by cervids in selected articles

Phytochemical group	Positive relationships	Negative relationships	Specificities
Phenolics	12/54	27/54	Three articles with both positive and negative relationships, depending on the subgroup of phenolic considered [81, 98 and 122].
Total phenolics	0/14	4/14	
Tannins	10/42	19/42	57% of studies are captive trial, 75% of captive trials used within species comparisons. 50% of articles investigated condensed tannins, only 8 articles investigated hydrolyzable tannins (6 with a negative relationship [42, 44, 45, 47, 52, 81]). Two studies report both a positive and a negative relationship [81, 122] although several report threshold in consumption (see result section).
Phenolic glycosides	0/10	9/10	All articles used within-species comparisons; 7/9 articles in natural environment. Specific glycosides considered: salicortin and tremulacin [39, 61, 106], salicin [61]. Articles [74] and [102] also considers several specific glycosides. Other articles consider total glycosides or several subgroups.
Other phenolics			Other phenolics studied with no reported relationships to consumption: salicylates [44, 98], non-tannin phenolics [68, 78], phenolics soluble, phenolics esterified, phenolics soluble potentially bound to cell walls [79]. Articles including several groups and specific phenolics: 73, 74, 102.
Flavonoids	0/6	4/6	Flavonoids investigated: several specific compounds and groups of compounds, including catechins and quercetin [73, 74, 112], specific flavonols, flavones and flavan-3-ols [102], total flavonoids [85, 112], flavanols, leucoanthocyanins [98].
Phenolic acids	5/6	0/6	Phenolic acids studied: chlorogenic acid [97, 98, 102, 103, 112], shikimic acid [111], several specific phenolics [111, 112].
Terpenes	3/37	29/37	70% of studies concerned coniferous plants. Only 7 studies made exclusively among-species comparisons.
Total terpenes	0/10	6/10	Total essential oils also included in this category. Two articles measured but did not test specific terpenes because of their high correlation with total terpene content [56, 57].
Groups of terpenes	3/24	15/24	45% of studies investigating total monoterpenes report negative relationships (9/20; one positive relationship [132]). Negative relationships also reported with sesquiterpenes lactones [50, 96], sesquiterpenes [101], oxygen-containing sesquiterpenes [92], diterpene acids [64, 84, 92], non-volatile crude terpenes [96]; Positive relationship with monoterpene content [132] and diterpenes [79,83]. Only articles investigating a group of triterpenes (saponins) reported no relationship [68].
Specific terpenes	0/24	17/24	Several specific terpenes, mostly monoterpenes, but some sesquiterpenes, diterpenes and triterpenes (see Online Resource 2 for complete list).
Resin	1/5	2/5	All articles but one with *Alces alces* (other cervid: *Odocoileus virginianus*).
Fibre	11/50	22/50	Three articles with positive and negative relationships [86, 112, 129]. 45 of the 50 articles include woody plants.

Articles are classified in groups of chemicals and in positive and negative relationships, based on summary realized during the systematic review. Classification in positive and negative relationship is a simplification of the results (see Online Resource 2 for a detailed summary of each article)

broad-leaved species (*Betula* spp., *Populus* spp., *Alnus viridis crispa, Salix* spp., *Eleagnus commutata, Sherphedia canadensis*) and one conifer species (*Picea glauca*). In one study [*17*], the application of resin from four broad-leaved species (*Betula papyrifera, Populus tremuloides, Populus balsamifera* and *Alnus viridis crispa*) reduced consumption, even though resin increased the content in nitrogen and phosphorus. The repellent effect of resin observed in [*27, Betula neoalaskana*] was attributed to a single compound, papyriferic acid (triterpene), while another constituent of resin, β-sitosterol, was not related to plant resistance.

Cervids Negative relations between consumption and resin content of *Betula* spp., are reported in two articles (Table 5). In [*113*], the relationship with *Pinus sylvestris* resin was also negative but not statistically significant. The positive relationship in [*131*] between ether extract in *Betula* sp. and consumption was explained by food selection for nitrogen content.

Fibre

In the selected articles, fibre content was sometimes reported in its three constituents (hemicellulose, cellulose and lignin), but a large proportion of articles used the Van Soest et al. (1991)'s method and separated fibre in fractions: neutral detergent fibre (NDF; hemicellulose, cellulose, lignin), acid detergent fibre (ADF; cellulose and lignin) and acid detergent lignin (ADL; lignin). The results reported did not seem to be influenced by the component of fibre content measured, as relationships were found with all components.

Leporids Six articles found negative relationships between consumption and fibre content (Table 4). In three articles, the low-fibre option was also the high-protein option [*7*, *9*, *13*], thus confounding the actual effect of fibre. Two articles found a positive relationship between consumption and digestible fibre (hemicellulose [*4*, *7*] or cellulose [*7*]), although the relationship was not significant in [*4*].

Cervids Of the 50 articles that reported measurement of fibre, 30 reported a relationship with consumption, predominantly negative relationships with one or more fibre-related variables (Table 5). In three studies, there was a significant effect of some fibre fractions but not lignin [*41, 71, 79*], which could be explained by a low variation in lignin content among forage options. Twelve articles reported positive relationships with fibre content, although these positive relationships can usually be explained by the avoidance of another phytochemical [*65, 74, 120*] or by food selection for another nutritional component [*121, 127*]. Two articles found a positive relationship between consumption and hemicellulose [*86, 112*], but two articles reported a negative relationship with hemicellulose [*84, 112*].

Discussion

Effects of Non-nutritive Phytochemicals

Using a systematic review approach, we synthesized 50 years of studies investigating the impacts of non-nutritive phytochemicals (secondary metabolites and fibre) on plant resistance to leporids and cervids. The 133 articles reviewed showed a large variety of study designs, and of plants and mammals studied, although they are mostly constrained to North America and Europe. The amount of research effort devoted to phytochemical groups varied and simple measures were widely used, yet, many researchers investigated specific phytochemicals. Based on this synthesis, we identified patterns in phytochemical effects on plant resistance for several phytochemical groups and subgroups.

In general, phenolics were related to a higher plant resistance to leporids and cervids, but these effects varied among specific groups of phenolics (e.g. phenolic glycosides, flavonoids, stilbenoids). Phenolic glycosides in particular seemed linked with plant resistance to cervids in natural environments. For leporids, the limited data available suggested flavonoids and stilbenoids increased plant resistance, but this interpretation bears further examination. Flavonoids also seemed to increased resistance to cervids, while phenolic acids decreased plant resistance. Terpenes were more consistently linked with higher plant resistance to both leporids and cervids than phenolics. This relationship differed between the two types of herbivores: it appeared driven by the overall terpene concentration and composition for cervids while for leporids, specific terpenes appeared to indicate higher plant resistance (e.g. 1,8-cineole). Cervids and leporids could differ in their terpene detoxification efficiency, either because their microbes differ in ability to transform terpenes (Kohl et al. 2018), or because their behaviour differs as rumination eliminates some volatile terpenes (Estell 2010). Rather than a general difference between herbivore families, diet breath could explain differences in terpene consumption, as specialists and generalists differ in terpene detoxification efficiency (Dearing et al. 2000; Shipley et al. 2012).

On the other hand, tannins and fibre were not good predictors of plant resistance. The effect of tannins on resistance appeared weak and inconsistent, probably because of the large assortment of anti-tannin strategies possessed by both herbivore families. Hindgut fermenters such as leporids could reduce the effects of tannins on the caecum microorganisms by chemical digestion (Cork and Foley 1991) and cecotrophy (feces consumption) could reduce the negative effect of tannin on nitrogen availability by allowing access to excreted microbial proteins and amino acids (Cork and Foley 1991). Cervids are also equipped to cope with tannins, as some ruminal microorganisms can tolerate tannins, and the ruminal pH promotes the bonding of tannins with several organic compounds (McSweeney et al. 2001). Moreover, concentrate selectors (e.g., *Alces alces*, *Odocoileus virginianus* and *O. hemionus*) and intermediate feeders (e.g., *Dama dama, Cervus elaphus*) are known for their saliva tannin-binding proteins, which reduces the negative impacts of tannins on digestibility (Robbins et al. 1987a). Because tannins reduced the amount of digestible proteins, their avoidance is probably low when the animals have sufficient access to nitrogen in their forage [e.g., *37, 71*] but their effect can be important when nitrogen is limiting. For example, McArt et al. (2009) compared the nutritional value of forage in two regions occupied by *Alces alces*. Tannin reduced, on average, protein digestibility by 46% and potentially led to nitrogen limitation in one region. Our review also suggests that cervids might require a minimal tannin intake: several of the reviewed articles reported consumption of high-tannin forage when low-tannin options were available or a selection of forage with an intermediate tannin content (see section 1.1). Tannins can exert a positive effect on the microbiota (McSweeney et al. 2001) and low concentration of condensed tannins can improve feeding value by reducing methane emission and increasing amino acid absorption (Jonker and Yu 2017).

Both herbivore types tended to avoid fibre, probably because even if they can digest them, their use of forage is not maximal under a high fibre content. Leporids have lower fibre digestion abilities than ruminants (Penry and Jumars 1987), and ruminants vary in their ability to digest fibre (Hofmann 1989). This ability is lower in concentrate selectors and intermediate feeders (the only ruminant feeding types in this review) than in roughage eaters (e.g. cattle). As for tannins, intake of fibre seemed to be regulated to achieve a constant fibre intake [see *8, 13, 16* for leporids] or the avoidance of fibre was subordinate to the acquisition of protein and nutritional compounds [see *54, 82, 86, 112, 127* for cervids]. Testing the effect of fibre on plant resistance is complicated by the fact fibre often covaries with other nutritional components. For example, the low-fibre option was also the high-protein option [*40, 80, 119, 124, 126, 129, 131*] or fibre content was positively correlated to forage abundance [*120, 121*]. In this context, the protein:fibre ratio could more accurately describe the effect of fibre on plant resistance [e.g., *86* and *130*]. An abundant literature on primates has demonstrated a positive selection for high protein:fibre ratios in plants, although contrary results exist (Felton et al. 2009). The use of protein:fibre ratio, however, is critiqued, notably because fibre estimates are biased with tannin-rich samples (Makkar et al. 1995; Wallis et al. 2012). Available nitrogen, although not evaluated in the articles reviewed, could be a good replacement measure (DeGabriel et al. 2009; McArt et al. 2009; Wallis et al. 2012). This measure is correlated to protein:fibre ratios, but takes into account the effect of tannins on nitrogen availability (Wallis et al. 2012).

The most prevalent pattern observed across all phytochemical groups was the high variability in the effects of non-nutritive phytochemicals on herbivore consumption. Results were less variable among specific groups or specific compounds, probably because narrowly defined groups of chemicals share chemical structure and function (e.g. Marsh et al. 2019). It is essential to note that factors such as the plant nutritional value and herbivore's condition and experience, for example, will modify herbivore food choices and their willingness to consume toxic and anti-nutritive compounds (Provenza et al. 2003; Raubenheimer et al. 2009). Consequently, plant resistance will be more variable in natural environments than in controlled conditions. Results that contradicted the consensus, however, often had a biological explanation. For example, a positive relationship between consumption and the concentration of a non-nutritive phytochemical can be explained by a food choice optimizing the intake of other nutritional constituents (e.g. nitrogen, see *40*). In general, the study design did not seem to influence the results reported, suggesting that the different experimental approaches have not induced bias. In a few studies, we consider that the ability of the authors to detect relationships could have been impeded by low samples sizes or the absence of sufficient variation in phytochemical concentrations.

Systematic reviews can be affected by publication bias, i.e. a higher probability of finding statistically significant effects in published literature, because studies without statistically significant results remain unpublished (Gurevitch and Hedges 1999). Our review could be biased against plants perceived as non-food [i.e. *Picea glauca* in 115] or phytochemicals recognized as highly toxic (i.e. alkaloids, see result section). However, the variability in observed responses, and the high frequency of articles not reporting relationships between phytochemicals and consumption (Tables 4, 5) suggested our systematic review has suffered little publication bias. Moreover, there is little evidence of publication bias in ecology (Jennions et al. 2013; Koricheva 2003), potentially because nonsignificant results are published alongside significant ones (Jennions et al. 2013). This seems to be the case for our selected articles: the effects of phytochemicals on consumption were often only one aspect of the article, included among other measures.

Studying Plant Resistance: What Should we Measure?

With so many phytochemicals involved in plant resistance and the high variability in relationships reported, we propose that the choice of chemical analyses must be tightly tailored to research objectives (e.g. evolutionary studies vs applied studies). Researchers interested in diet selection processes and the ecology and evolution of plant resistance should consider several nutritional drivers concurrently (Felton et al. 2018; Wam et al. 2017), as proposed in nutritional frameworks such as the Geometric Framework (Raubenheimer et al. 2009). Therefore, these questions should be tackled with a thorough approach examining specific compounds, as the effect of phytochemicals on herbivore food choices seems more tightly linked to individual compounds and narrowly defined groups of phytochemicals (Felton et al. 2018). Special interest should be given to understudied phytochemicals, such as flavonoids and stilbenoids, as our review found these were often connected to herbivore consumption. Metabolomic techniques, i.e. the untargeted analysis of a wide range of metabolites within a sample (Macel et al. 2010), could also be used to answer ecological and evolutionary questions. These techniques are used in plant-herbivore studies to compare plant resistance within species (Bundy et al. 2009) but must be combined with further assays to identify specific phytochemicals associated with resistance (Macel et al. 2010). Using either conventional or metabolomics techniques, analyses including several compounds allow the evaluation of potential synergistic or additive effects among phytochemicals (Richards et al. 2016; Stolter et al. 2013). Synergistic and additive effects could emerge if several phenolics are detoxified by a single metabolic pathway, an explanation known as the detoxification limitation hypothesis (Freeland and Janzen 1974). According to this hypothesis, feeding is constrained by the rate of detoxification of phytochemicals (Marsh et al. 2006). Consequently, if several phytochemicals are detoxified by the same pathway, their combined effect will synergistically reduce feeding by herbivores. Synergistic effects of mixtures of phytochemical could also arise through toxicity on several metabolic functions (Richards et al. 2016). Without question, investigations of the ecology and evolution of plant resistance need to take into account the effects of mixtures.

When the study objective is to compare plant resistance within an applied field, a more parsimonious approach could be considered based on the proxies of plant resistance identified in this review. These recommendations can apply for both types of herbivores, as our synthesis showed more similarities than differences in plant resistance to them. For within-species comparisons of resistance, crude measures such as resin content and total phenolics could be used, although they are not always related to plant resistance. These proxies should be used with care, and are best avoided when comparing among species as these methods can be biased. For example, total phenolics measurement by Folin-type assays are known to be biased when the phenolic composition differ, making among species comparison hazardous (Appel et al. 2001). To compare resistance potential among species, more specific groups of phenolics or of terpenes, such as flavonoids, stilbenoids or monoterpenes should be favoured. For researchers interested in resistance to leporids, the concentration of specific compounds avoided by these herbivores (e.g. 1,8-cineole, pinosylvin) could be quantified, when known to be present in the plant of interest. Finally, measurement of available nitrogen (DeGabriel et al. 2009; McArt et al. 2009; Wallis et al. 2012) should be preferred over measures of tannins or fibre, because this measure takes into account the nutritional value of the plant, known to modulate the effect of tannins and fibre on plant resistance.

Diet composition has often been considered as a fixed and relatively stable component of herbivores' foraging process, which allowed the classification of plants as preferred or avoided forage. We are now aware, however, that herbivore resource selection varies in space and time. This review highlights the potential effects of intraspecific variation in phytochemistry on resource selection and on plant resistance to herbivores. These effects could be magnified in the future, as climate change can modify plant investment in phytochemicals (Zvereva and Kozlov 2006), with consequences for plant-herbivore interactions. An improved consideration of plant defence, based on multiple and meaningful chemical analyses could be essential to predict novel or changing plant-herbivore interactions.

Acknowledgments EC was supported by a Mitacs Accelerate Fellowship, resulting from a partnership among Ouranos, Université Laval and Direction de la recherche forestière (Ministère des Forêts, de la Faune et des Parcs). We thank B. Carrier, P. Edwards, J. Wiedenbeck, A. Sledgers and two anonymous reviewers for revising previous versions of this article, S. Béland for the silhouettes of Fig. 2, and D. Houle and A. D. Munson for their advices.

References

Adams RP, Muir JP, Taylor CA, Whitney TR (2013) Differences in chemical composition between browsed and non-browsed *Juniperus ashei* Buch. Trees. Biochem Syst Ecol 46:73–78. https://doi.org/10.1016/j.bse.2012.09.020

Agrawal AA, Weber MG (2015) On the study of plant defence and herbivory using comparative approaches: how important are secondary plant compounds. Ecol Lett 18:985–991. https://doi.org/10.1111/ele.12482

Anderson KE, Inouye BD, Underwood N (2015) Can inducible resistance in plants cause herbivore aggregations? Spatial patterns in an inducible plant/herbivore model. Ecology 96:2758–2770. https://doi.org/10.1890/14-1697.1

Appel HM, Govenor HL, D'ascenzo M, Siska E, Schultz JC (2001) Limitations of Folin assays of foliar phenolics in ecological studies. J Chem Ecol 27:761–778

Averill KM, Mortensen DA, Smithwick EA, Post E (2016) Deer feeding selectivity for invasive plants. Biol Invasions 18:1247–1263. https://doi.org/10.1007/s10530-016-1063-z

Ayres MP, Clausen TP, MacLean SF, Redman AM, Reichardt PB (1997) Diversity of structure and antiherbivore activity in condensed tannins. Ecology 78:1696–1712. https://doi.org/10.1890/0012-9658(1997)078[1696:dosaaa]2.0.co;2

Bernays EA, Driver GC, Bilgener M (1989) Herbivores and plant tannins. Adv Ecol Res 19:263–302. https://doi.org/10.1016/S0065-2504(08)60160-9

Bundy J, Davey M, Viant M (2009) Environmental metabolomics: a critical review and future perspectives. Metabolomics 5:3–21. https://doi.org/10.1007/s11306-008-0152-0

Carmona D, Lajeunesse MJ, Johnson MTJ (2011) Plant traits that predict resistance to herbivores. Funct Ecol 25:358–367. https://doi.org/10.1111/j.1365-2435.2010.01794.x

Chamberlain S et al. (2018) Taxize: taxonomic information from around the web. R package version 0.9.3. https://github.com/ropensci/taxize

Chapman GA, Bork EW, Donkor NT, Hudson RJ (2010) Effects of supplemental dietary tannins on the performance of white-tailed deer (*Odocoileus virginianus*). J Anim Physiol Anim Nutr 94:65–73. https://doi.org/10.1111/j.1439-0396.2008.00883.x

Constabel CP (1999) A survey of herbivore-inducible defensive proteins and phytochemicals. In: Agrawal AA, Tuzun S, Bent E (eds) Induced plant defenses against pathogens and herbivores. The American Phytopathological Society, St. Paul, pp 137–166

Cork SJ, Foley WJ (1991) Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperate and tropical forests. In: Palo RT, Robbins CT (eds) Plant defenses against mammalian herbivory. CRC Press Inc., Boca Raton, pp 133–166

Côté SD, Rooney TP, Tremblay J-P, Dussault C, Waller DM (2004) Ecological impacts of deer overabundance. Annu Rev Ecol Evol S 35:113–147. https://doi.org/10.1146/annurev.ecolsys.35.021103.105725

de Garine-Wichatitsky M, Duncan P, Suprin B, Chardonnet P, Maillard D (2003) A review of the diet of Rusa deer *Cervus timorensis russa* in New Caledonia: are the endemic plants defenceless against this introduced, eruptive ruminant? Pac Conserv Biol 9:136–143. https://doi.org/10.1071/PC030136

Dearing MD, Mangione AM, Karasov WH (2000) Diet breadth of mammalian herbivores: nutrient versus detoxification constraints. Oecologia 123:397–405. https://doi.org/10.1007/s004420051027

DeGabriel JL, Moore BD, Foley WJ, Johnson CN (2009) The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology 90:711–719. https://doi.org/10.1890/08-0940.1

DeGabriel JL et al (2014) Translating nutritional ecology from the laboratory to the field: milestones in linking plant chemistry to population regulation in mammalian browsers. Oikos 123:298–308. https://doi.org/10.1111/j.1600-0706.2013.00727.x

Estell RE (2010) Coping with shrub secondary metabolites by ruminants. Small Ruminant Res 94:1–9. https://doi.org/10.1016/j.smallrumres.2010.09.012

Feeny P (1991) The evolution of chemical ecology: contributions from the study of herbivorous insects. In: Rosenthal GA, Berenbaum MR (eds) Herbivores, Their interactions with secondary plant metabolites, vol 2. Academic Press Inc., San Diego, pp 1–44

Felton AM, Felton A, Lindenmayer DB, Foley WJ (2009) Nutritional goals of wild primates. Funct Ecol:70-78. https://doi.org/10.1111/j.1365-2435.2008.01526.x

Felton AM, Wam HK, Stolter C, Mathisen KM, Wallgren M (2018) The complexity of interacting nutritional drivers behind food selection, a review of northern cervids. Ecosphere 9:e02230. https://doi.org/10.1002/ecs2.2230

Fraenkel GS (1959) The raison d'être of secondary plant substances. Science 129:1466–1470. https://doi.org/10.1126/science.129.3361.1466

Freeland WJ, Janzen DH (1974) Strategies in herbivory by mammals: the role of plant secondary compounds. Am Nat 108:269–289

Gershenzon J, Croteau R (1991) Terpenoids. In: Rosenthal GA, Berenbaum MR (eds) Herbivores. Their interactions with secondary plant metabolites, vol 1. 2nd edn. Academic Press Inc, San Diego, pp 165–220

Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3:408–414. https://doi.org/10.1038/nchembio.2007.5

Gurevitch J, Hedges LV (1999) Statistical issues in ecological meta-analyses. Ecology 80:1142–1149. https://doi.org/10.1890/0012-9658(1999)080[1142:siiema]2.0.co;2

Hagerman AE, Butler LG (1991) Tannins and lignins. In: Rosenthal GA, Berenbaum MR (eds) Herbivores. Their interactions with secondary plant metabolites, vol volume 1: the chemical participants. Academic Press Inc, San Diego, pp 355–388

Hammerschmidt R, Smith-Becker JA (1999) The role of salicylic acid in disease resistance. In: Agrawal AA, Tuzun S, Bent E (eds) Induced plant defenses against pathogens and herbivores. The American Phytopathological Society, St. Paul, pp 37–53

Harborne JB (1991) The chemical basis of plant defense. In: Palo RT, Robbins CT (eds) Plant defenses against mammalian herbivory. CRC Press Inc., Boca Raton, pp 45–60

Hester AJ, Bergman M, Iason GR, Moen J (2006) Impacts of large herbivore on plant community structure and dynamics. In: Danell K, Bergström R, Duncan P, Pastor J (eds) Large herbivore ecology, ecosystem dynamics and conservation. Cambridge University Press, Cambridge, pp 97–141

Hirakawa H (2001) Coprophagy in leporids and other mammalian herbivores. Mamm Rev 31:61–80. https://doi.org/10.1046/j.1365-2907.2001.00079.x

Hofmann RR (1989) Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78:443–457

Holeski LM, McKenzie SC, Kruger EL, Couture JJ, Rubert-Nason K, Lindroth RL (2016) Phytochemical traits underlie genotypic variation in susceptibility of quaking aspen (*Populus tremuloides*) to browsing by a keystone forest ungulate. J Ecol 104:850–863. https://doi.org/10.1111/1365-2745.12559

Iason G (2005) The role of plant secondary metabolites in mammalian herbivory: ecological perspectives. Proc Nutr Soc 64:123–131. https://doi.org/10.1079/PNS2004415

Iason GR, Villalba JJ (2006) Behavioral strategies of mammal herbivores against plant secondary metabolites: the avoidance-tolerance continuum. J Chem Ecol 32:1115–1132. https://doi.org/10.1007/s10886-006-9075-2

Jennions MD, Lortie CJ, Rosenberg MS, Rothstein HR (2013) Publication and related biases. In: Koricheva J, Gurevitch J, Mengeresen K (eds) Handbook of meta-analysis in ecology and evolution. Princeton University Press, Princeton, pp 207–236

Jones CG, Firn RD, Malcolm SB (1991) On the evolution of plant secondary chemical diversity. Philos T Roy Soc B 333:273–280. https://doi.org/10.1098/rstb.1991.0077

Jonker A, Yu PQ (2017) The occurrence, biosynthesis, and molecular structure of proanthocyanidins and their effects on legume forage protein precipitation, digestion and absorption in the ruminant digestive tract. Int J Mol Sci 18. https://doi.org/10.3390/ijms18051105

Kohl KD et al (2018) Metagenomic sequencing provides insights into microbial detoxification in the guts of small mammalian herbivores (Neotoma spp.). FEMS Microbiol Ecol 94. https://doi.org/10.1093/femsec/fiy184

Koricheva J (2003) Non-significant results in ecology: a burden or a blessing in disguise? Oikos 102:397–401

Kumar S, Pandey AK (2013, 2013) Chemistry and biological activities of flavonoids: an overview. Sci World J. https://doi.org/10.1155/2013/162750

Laca EA, Demment MW (1996) Foraging strategies of grazing animals. In: Hodgson J, Illius AW (eds) The ecology and management of grazing systems. CAB International, Wallingford, pp 137–158

Laca EA, Shipley LA, Reid ED (2001) Structural anti-quality characteristics of range and pasture plants. J Range Manage 54:413–419. https://doi.org/10.2307/4003112

Lindroth RL (2000) Adaptations of quaking aspen for defense against damage by herbivores and related environmental agents. USDA Forest Service proceedings RMRS-P-18

Liu XJ, Vrieling K, Klinkhamer PGL (2017) Interactions between plant metabolites affect herbivores: a study with pyrrolizidine alkaloids and chlorogenic acid. Front Plant Sci 8. https://doi.org/10.3389/fpls.2017.00903

Loison A, Gaillard J, Pelabon C, Yoccoz N (1999) What factors shape sexual size dimorphism in ungulates? Evol Ecol Res 1:611–633

Macel M, van Dam NM, Keurentjes JJ (2010) Metabolomics: the chemistry between ecology and genetics. Mol Ecol Resour 10:583–593. https://doi.org/10.1111/j.1755-0998.2010.02854.x

Majak W (2001) Review of toxic glycosides in rangeland and pasture forages. J Range Manage:494–498

Makkar H, Borowy N, Becker K, Degen A (1995) Some problems in fiber determination of a tannin-rich forage (*Acacia saligna* leaves) and their implications in *in vivo* studies. Anim Feed Sci Technol 55:67–76. https://doi.org/10.1016/0377-8401(95)00793-M

Malhotra B, Onyilagha J, Bohm B, Towers G, James D, Harborne J, French C (1996) Inhibition of tomato ringspot virus by flavonoids. Phytochemistry 43:1271–1276

Mali S, Borges R (2003) Phenolics, fibre, alkaloids, saponins, and cyanogenic glycosides in a seasonal cloud forest in India. Biochem Syst Ecol 31:1221–1246. https://doi.org/10.1016/S0305-1978(03)00079-6

Marsh KJ, Wallis IR, Andrew RL, Foley WJ (2006) The detoxification limitation hypothesis: where did it come from and where is it going? J Chem Ecol 32:1247–1266. https://doi.org/10.1007/s10886-006-9082-3

Marsh KJ, Wallis IR, Kulheim C, Clark R, Nicolle D, Foley WJ, Salminen JP (2019) New approaches to tannin analysis of leaves can be used to explain in vitro biological activities associated with herbivore defence. New Phytol. https://doi.org/10.1111/nph.16117

McArt SH, Spalinger DE, Collins WB, Schoen ER, Stevenson T, Bucho M (2009) Summer dietary nitrogen availability as a potential bottom-up constraint on moose in south-Central Alaska. Ecology 90:1400–1411. https://doi.org/10.1890/08-1435.1

McArthur C, Hagerman AE, Robbins CT (1991) Physiological strategies of mammalian herbivores against plant defenses. In: Palo RT, Robbins CT (eds) Plant defenses against mammalian herbivory. CRC Press Inc., Boca Raton, pp 103–114

McSweeney C, Palmer B, McNeill D, Krause D (2001) Microbial interactions with tannins: nutritional consequences for ruminants. Anim Feed Sci Technol 91:83–93. https://doi.org/10.1016/S0377-8401(01)00232-2

Moore BD, Lawler IR, Wallis IR, Beale CM, Foley WJ (2010) Palatability mapping: a koala's eye view of spatial variation in habitat quality. Ecology 91:3165–3176. https://doi.org/10.1890/09-1714.1

Moore BD, Andrew RL, Külheim C, Foley WJ (2014) Explaining intraspecific diversity in plant secondary metabolites in an ecological context. New Phytol 201:733–750. https://doi.org/10.1111/nph.12526

Nagy JG, Tengerdy RP (1968) Antibacterial action of essential oils of *Artemisia* as an ecological factor. II. Antibacterial action of the volatile oils of *Artemisia tridentata* (big sagebush) on bacteria from the rumen of mule deer. Appl Microbiol 16:441–444

Oldemeyer JL (1983) Browse production and its use by moose and snowshoe hares at the Kenai moose research center, Alaska. J Wildl Manag 47:486–496. https://doi.org/10.2307/3808521

Penry DL, Jumars PA (1987) Modeling animal guts as chemical reactors. Am Nat 129:69–96. https://doi.org/10.1086/284623

Picman AK (1986) Biological activities of sesquiterpene lactones. Biochem Syst Ecol 14:255–281. https://doi.org/10.1016/0305-1978(86)90101-8

Picman J, Picman AK, Towers GHN (1982) Effects of the sesquiterpene lactone, helenin, on feeding rates and survival of the tundra redback vole *Clethrionomys rutilus*. Biochem Syst Ecol 10:269–273. https://doi.org/10.1016/0305-1978(82)90041-2

Provenza FD, Villalba JJ, Dziba LE, Atwood SB, Banner RE (2003) Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Res 49:257–274. https://doi.org/10.1016/S0921-4488(03)00143-3

Raguso RA, Agrawal AA, Douglas AE, Jander G, Kessler A, Poveda K, Thaler JS (2015) The raison d'être of chemical ecology. Ecology 96:617–630. https://doi.org/10.1890/14-1474.1

Raubenheimer D, Simpson SJ, Mayntz D (2009) Nutrition, ecology and nutritional ecology: toward an integrated framework. Funct Ecol 23:4–16. https://doi.org/10.1111/j.1365-2435.2009.01522.x

Richards LA, Glassmire AE, Ochsenrider KM, Smilanich AM, Dodson CD, Jeffrey CS, Dyer LA (2016) Phytochemical diversity and synergistic effects on herbivores. Phytochem Rev 15:1153–1166. https://doi.org/10.1007/s11101-016-9479-8

Robbins C, Mole S, Hagerman A, Hanley T (1987a) Role of tannins in defending plants against ruminants: reduction in dry matter digestion? Ecology:1606-1615. https://doi.org/10.2307/1939852

Robbins CT, Hanley TA, Hagerman AE, Hjeljord O, Baker DL, Schwartz CC, Mautz WW (1987b) Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68:98–107

Schwartz CC, Nagy JG, Regelin WL (1980) Juniper oil yield, terpenoid concentration, and antimicrobial effects on deer. J Wildl Manag 44:107–113. https://doi.org/10.2307/3808356

Semiadi G, Barry T, Muir PD, Hodgson J (1995) Dietary preferences of sambar (*Cervus unicolor*) and red deer (*Cervus elaphus*) offered browse, forage legume and grass species. J Agric Sci 125:99–107. https://doi.org/10.1017/S0021859600074554

Shipley LA, Davis EM, Felicetti LA, McLean S, Forbey JS (2012) Mechanisms for eliminating monoterpenes of sagebrush by specialist and generalist rabbits. J Chem Ecol 38:1178–1189. https://doi.org/10.1007/s10886-012-0192-9

Steuer P, Sudekum KH, Muller DWH, Kaandorp J, Clauss M, Hummel J (2013) Fibre digestibility in large herbivores as related to digestion type and body mass - an in vitro approach. Comp Biochem Phys A 164:319–326. https://doi.org/10.1016/j.cbpa.2012.10.026

Stolter C, Ball JP, Julkunen-Tiitto R (2013) Seasonal differences in the relative importance of specific phenolics and twig morphology result in contrasting patterns of foraging by a generalist herbivore. Can J Zool 91:338–347. https://doi.org/10.1139/cjz-2012-0270

Tahvanainen J, Helle E, Julkunentiitto R, Lavola A (1985) Phenolic compounds of willow bark as deterrents against feeding by mountain hare. Oecologia 65:319–323. https://doi.org/10.1007/bf00378905

Taiz L, Zeiger E (eds) (2002) Plant physiology, 3rd edn. Sinauer Associates, Inc., Sunderland

Underwood N, Anderson K, Inouye BD (2005) Induced vs. constitutive resistance and the spatial distribution of insect herbivores among plants. Ecology 86:594–602. https://doi.org/10.1890/03-0290

Van Soest P, Robertson J, Lewis B (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597

Wallis IR et al (2012) Food for folivores: nutritional explanations linking diets to population density. Oecologia 169:281–291. https://doi.org/10.1007/s00442-011-2212-9

Wam HK, Felton AM, Stolter C, Nybakken L, Hjeljord O (2017) Moose selecting for specific nutritional composition of birch places limits on food acceptability. Ecol Evol 8. https://doi.org/10.1002/ece3.3715

Zvereva E, Kozlov M (2006) Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: a metaanalysis. Glob Chang Biol 12:27–41. https://doi.org/10.1111/j.1365-2486.2005.01086.x