UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRADA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Dissertação de Mestrado

Papel de derivados fenólicos foliares no comportamento alimentar de *Porcellio scaber* (Isopoda: Oniscidea)

ROSANA APARECIDA ARENHARDT MARIANI

Porto Alegre, Maio de 2019

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Mestre em Ecologia.

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RESUMO

Os derivados fenólicos desempenham importante papel na defesa contra hebivoria e também podem influenciar o comportamento dos organismos decompositores. O objetivo principal do presente trabalho foi verificar a influência da variação do teor de fenólicos totais e flavonoides totais presentes em diferentes estágios de decomposição foliar de *Carya illinoinensis* (nogueira pecan) no comportamento alimentar (taxas de consumo, de assimilação e egestão) de *Porcellio scaber* (Isopoda: Oniscidea). Investigou-se também a influência isolada (alimento artificial) de dois flavonoides, rutina (3-orutinosilquercetina) e quercetina, no comportamento alimentar deste isópodo. Folhas verdes de *C. illinoinensis* foram submetidas ao processo de decomposição em *litter bags* em condições ambientais por até três meses. Folhas verdes, folhas com um, dois, e três meses de decomposição (tratamentos) foram oferecidas aos isópodos em experimento sem escolha. Foram quantificados os teores de derivados fenólicos totais e de flavonoides totais nas folhas de cada período avaliado. Discos de gel de agarose com rutina e quercetina (dieta artificial) em diferentes concentrações (0,0; 0,5; 1,0 e 2,0 mg/ml) também foram usados como tratamentos em experimentos de comportamento alimentar com *P. scaber*. A taxa de consumo não diferiu estatisticamente entre os estágios de decomposição de folhas de *C. illinoinensis* ($H = 6.3059$, $P = 0.0976$). A taxa de egestão não diferiu significativamente ($H = 6,6857$, $P = 0,0826$). A taxa de assimilação das folhas de três meses diferiu das folhas verdes e das folhas com um mês de decomposição, mas não diferiu das folhas com dois meses de decomposição. O teor de derivados fenólicos totais e flavonoides totais diferiram significativamente entre todos os estágios avaliados ($H = 9,6525$, $P = 0.0218$ e $H = 10.3846$, $P = 0.0156$, respectivamente). Folhas verdes tiveram o maior teor de fenólicos totais ($185,441 \pm 0.58$ mg equivalentes de ácido gálico/g de folha seca) e flavonoides totais ($209,057 \pm 2,97$ mg equivalentes de rutina/g de folha seca). Nos experimentos com gel de agarose, rutina e quercetina mostraram toxidez considerável apenas na maior concentração utilizada (2,0 mg/ml), além de altas taxas de mortalidade para a concentração 2,0 mg/ml, mas a quercetina se mostrou mais tóxica. Não foi possível identificar uma concentração ótima que estimulasse o comportamento alimentar de *P. scaber*. O comportamento alimentar de *P. scaber* não foi inibido pela presença de alto teor de fenólicos totais e flavonoides totais presentes nas folhas de *C. illinoinensis*.

Palavras-chave: Isópodos terrestres, Porcellionidae, *Carya illinoinensis*, Decomposição foliar.

ABSTRACT

Phenolic derivatives play an important role in defense against herbivory and can also influence the behavior of decomposing organisms. The aims of the study were to verify the influence of total phenolic content and total flavonoid content presents in different stages of leaf decomposition of *Carya illinoinensis* (hickory) in feeding behavior (consumption, assimilation and egestion rates) of *Porcellio scaber* (Isopoda: Oniscidea). In was also investigated the isolated influence (artificial diet) of two flavonoids, rutin (3-orutinosylquercetin) and quercetin, on the feeding behavior of this isopod. Green leaves of *C. illinoinensis* were submitted to the decomposition process in litter bags under environmental conditions for up to three months. Green leaves, leaves with one, two, and three months of decomposition (treatments) were offered to the isopods in no choice experiment. The amount of total phenolic content and total flavonoid content in leaves were evaluated in each decomposition stage. Agarose gel disks with rutin and quercetin (artificial diet) at different concentrations (0.0, 0.5, 1.0 and 2.0 mg / ml) were also used as treatments in *P. scaber* feeding behavior experiments. No statistical differences were found to egestion rates ($H = 6.6857$, $P = 0.0826$). The assimilation rate of three month old leaves differed from of green leaves and one month old leaves, but not from that of two month old leaves ($H = 18.2856$, $P = 0.0004$). The total phenolic content and total flavonoid content differed significantly among all evaluated decomposition stages ($H = 9.6525$, $P = 0.0218$ and $H = 10.3846$, $P = 0.0156$, respectively). Green leaves had the highest value of total phenolic content (185.441 ± 0.58 mg equivalent of gallic acid /mg dry weight) and total flavonoid content (209.057 ± 2.97 mg of rutin equivalents/g dry weight). In the experiments with artificial diet, rutin and quercetin shows toxicity only in high doses and induced the highest mortality rates at the concentration of 2.0 mg / ml. The high amount of total phenolic content and total flavonoid content mainly in leaves of *C. illinoinensis* don't repelled *P. scaber*. In the experiments with artificial diet, rutin and quercetin showed considerable toxicity only at the highest concentration used (2.0 mg/ml) and induced high mortality rates at 2.0 mg / ml, but quercetin showed a greater toxic potential than its glycosylated derivative. It was not possible to identify an optimum concentration that stimulated the feeding behavior of *P. scaber*. The feeding behavior of *P. scaber* was not inhibited by high total phenolic and total flavonoid contents in the *C. illinoinensis* leaves.

Key-words: Terrestrial isopods, Porcellionidae, *Carya illinoinensis*, Leaf decomposition.

SUMÁRIO

1 INTRODUÇÃO

A serapilheira presente nos ambientes terrestres é em grande parte constituída por material vegetal, sendo que as folhas representam grande parte do aporte nutricional deste material que se acumula na superfície do solo (MEGURO; VINUEZA; DELITTI, 1979; BEGON; TOWNSEND; HARPER, 2007). O processo de decomposição deste material vegetal é de extrema importância para o ciclo de nutrientes e fluxo de energia através do solo, pois é por intermédio da degradação da serapilheira que os nutrientes orgânicos presentes em restos animais e vegetais (imobilização) são convertidos para a forma inorgânica (mineralização) juntamente com liberação de energia (BEGON; TOWNSEND; HARPER, 2007). Entretanto, esse processo pode ser influenciado por fatores abióticos e bióticos, tais como a presença de metabólitos secundários de plantas, os quais podem retardar a perda de massa foliar (COQ *et al*., 2010); e por organismos do solo, uma vez que a presença destes indivíduos pode acelerar a decomposição foliar (GONZÁLEZ; SEASTEDT, 2001; COQ *et al*., 2010).

1.1 Metabólitos secundários

Os metabólitos secundários (substâncias fenólicas) são assim chamados por não participarem intrinsecamente de funções no metabolismo primário das plantas, ou seja, são produtos do metabolismo secundário (METCALF; METCALF, 1992; WATERMAN; MOLE, 1994). Entretanto, essas substâncias podem ser encontradas em várias partes das plantas, e atuam dando cor, cheiro e gosto aos materiais vegetais (METCALF; METCALF, 1992). A biossíntese destes metabólitos, como os derivados fenólicos (taninos hidrolisáveis, flavonoides incluindo os proantocianidinas) desempenham um papel fundamental no processo de sinalização, pois atuam de maneira positiva e negativa em relação a outros organismos vivos (HARBORNE, 1993). Uma importante função de sinalização desempenhada pelos derivados fenólicos é como barreira química, uma vez que atuam repelindo o consumo de vertebrados e invertebrados (devido gosto adstringente dos taninos) e minimizando o efeito do ataque de herbívoros e de patógenos nas plantas (HARBORNE, 1993; KEFERI; KALEVITCH; BORSARI, 2003). Esta sinalização das plantas para os herbívoros pode ser visual e/ou olfativa, servindo como um alerta antes mesmo que o consumo ocorra (HARBORNE, 1993). Entretanto, quando o consumo já ocorreu, a presença destas substâncias no alimento é capaz de afetar parâmetros populacionais (fecundidade) de insetos herbívoros (AWMACK; LEATHE, 2002).

Os flavonoides, polifenóis de anel benzopirano (WATERMAN; MOLE, 1994), são um grupo de sinalizadores particularmente versáteis. Estas substâncias são responsáveis pela coloração das flores (cores creme e branco: quercetina; amarelo: carotenoides) e pelas linhas de néctar, as quais sinalizam para os polinizadores e os conduzem até o néctar (HARBORNE, 1993). Além disso, sinalizam para a oviposição de insetos herbívoros, auxiliando o inseto no reconhecimento de uma planta hospedeira (HARBORNE, 1993; SIMMONDS, 2001). Estas substâncias também atuam na proteção contra diversos tipos de estresses, além disso, podem ser encontrados facilmente em folhas de muitas angiospermas (HARBORNE, 1993; SIMMONDS, 2001; BOUÉ; RAINA, 2003). Os flavonoides desempenham importante ação fagoinibidora para herbívoros (HARBORNE, 1993). Estudos mostram que os flavonoides monoméricos e seus polímeros (proantocianidinas, também denominadas de taninos condensados) possuem papel importante na interação entre herbívoros e plantas (SIMMONDS, 2001; BOUÉ; RAINA, 2003) e podem apresentar efeito tóxico em insetos (METCALF; METCALF, 1992; HARBORNE, 1993). Entretanto, o efeito tóxico dependerá da dose, tempo de exposição e animal utilizado no estudo (HARBORNE, 1993). Em estudo realizado por Rauha *et al*. (2000), extrato vegetal contendo o flavonoide quercetina foi eficaz na inibição do crescimento de todos os microrganismos procariotos estudados (*Staphylococcus* sp., *Bacillus* sp., *Micrococcus* sp., *Escherichia* sp.), enquanto que rutina, molécula de quercetina glicosilada, permaneceu inativa, não afetando os organismos. Registros mostram que alguns flavonoides (rutina e isoquercetrina) são capazes de modular negativamente o comportamento alimentar de herbívoros, reduzindo o consumo (HARBORNE, 1993; SIMMONDS, 2001). Essas substâncias também podem ser facilmente encontradas em outros materiais provenientes de plantas como folhas senescentes ou qualquer outro órgão vegetativo que compõem a serapilheira, nestes casos a liberação dos metabólitos (voláteis ou não) irá influenciar o comportamento dos organismos do solo, como os isópodos terrestres (MILLAR *et al*., 1986; ZIMMER, 2002).

1.2 Fauna do solo

A fauna edáfica é composta por organismos que habitam o solo. Estes organismos variam no tamanho (de microfauna a macrofauna) e podem ocorrem em diversos ambientes. Desempenham importante função ecológica, pois atuam na fragmentação do material foliar presente na serapilheira. É através da fragmentação realizada por estes organismos que os nutrientes presentes na matéria orgânica morta retornam para o solo e para o ciclo de nutrientes. Entretanto, a atividade destes organismos bem como sua contribuição para a mineralização, é fortemente influenciada pelas características do material vegetal, tais como presença de substâncias de defesa, dureza, lignina, entre outros (BEGON; TOWNSEND; HARPER, 2007). Acari, Coleoptera, Diplopoda, Gastropoda, Isopoda, Heteroptera, Hymenoptera, Diptera e Isoptera são alguns grupos taxonômicos normalmente encontrados no solo compondo a fauna edáfica (BEGON; TOWNSEND; HARPER, 2007; FERREIRA *et al*., 2017).

1.3 Isópodos terrestres

A Ordem Isopoda é a segunda maior do subfilo Crustacea com cerca de 10.000 (10 mil) espécies que vivem em ambiente marinho, dulcícola e terrestre, com indivíduos que variam de 0,5 mm a 500 mm de comprimento (RUPPERT; BARNES, 1996; MARTIN; DAVIS, 2007). A subordem Oniscidea, com cerca de 5.000 (cinco mil) espécies, compreende o único grupo no qual os crustáceos obtiveram sucesso para habitar o ambiente terrestre com total independência do meio aquático (MARTIN; DAVIS, 2007). Isto só foi possível devido algumas adaptações como: desenvolvimento direto, trocas gasosas devido à presença de pseudotraquéias, regulação osmótica, corpo mais achatado e cutícula espessa para evitar perda de água (RUPPERT; BARNES, 1996; MARTIN; DAVIS, 2007), outros fatores que contribuíram incluem hábito noturno, viver em locais mais úmidos e ter comportamento gregário (RUPPERT; BARNES, 1996).

Os oniscídeos, popularmente conhecidos como tatuzinhos-de-jardim, são artrópodos que vivem no solo e estão amplamente distribuídos tanto em regiões tropicais quanto em regiões temperadas (ARAUJO; TAITI, 2007; LISBOA *et al*., 2013). Habitam uma variedade de ambientes, e podem ser encontrados desde locais mais úmidos até áreas mais secas (ARAUJO; TAITI, 2007).

Como compõem os primeiros níveis tróficos de cadeias alimentares, podem servir como uma fonte de recurso alimentar para outros organismos predadores como aranhas, besouros e pequenos mamíferos (dentre outros organismos) (SUTTON, 1970). Os oniscídeos também desempenham importante função ecológica (HÄTTENSCHWILER; BRACHT-JORGENSEN, 2010; LISBOA *et al*., 2013), pois fragmentam a serapilheira durante seu consumo, aumentando a área de superfície exposta do material e, consequentemente, favorecem a colonização microbiana (OSLER; SOMMERKORN, 2007; ABD EL-WAKEIL, 2015). Além disso, a atividade detritívora aumenta a liberação de nutrientes para o solo (KAUTZ; TOPP, 2000; ZIMMER; KAUTZ; TOPP, 2005). Portanto, a atividade detritívora dos isópodos (e outros organismos do solo) contribui de forma significativa com a decomposição foliar, muitas vezes acelerando o processo através da transformação física e química da matéria orgânica do solo (GONZÁLEZ; SEASTEDT, 2001; ZIMMER *et al*., 2002a; ZIMMER; KAUTZ; TOPP, 2005; MEWER; OSTERTAG; COWIE, 2011; RIUTTA *et al*., 2012; WANG; YIN; LI, 2015).

Os isópodos terrestres possuem hábitos alimentares bem variados (saprófagos, onívoros), mas no geral consomem material vegetal em decomposição (folhas, restos vegetais, restos animais) (RUPPERT; BARNES, 1996; MARTIN; DAVIS, 2007). Apesar dos isópodos serem capazes de utilizar uma grande variedade de alimentos em processo de decomposição (LISBOA *et al*., 2013), tem-se evidenciado certo grau de preferência por algumas fontes (ZIMMER *et al*., 2002a; DAVID, 2014). Esse comportamento pode estar relacionado a diversos fatores, dentre eles a quantidade de nutrientes, a presença de metabólitos desagradáveis ou impalatáveis, grau de colonização microbiana, senescência e o grau de decomposição das folhas (HASSALL; TURNER; RANDS, 1987; AWMACK; LEATHE, 2002; ZIMMER; KAUTZ; TOPP, 2003). Estudos têm mostrado que a ocorrência de oniscídeos em determinados habitats, bem como distintos perfis comportamentais estão relacionados a características químicas de fontes alimentares. Esses atributos das plantas refletem na qualidade do alimento para os isópodos terrestres, pois quanto maior as concentrações de compostos recalcitrantes (ex.: fenólicos, lignina, dureza) na serapilheira, menor é a preferência alimentar e o consumo dos indivíduos detritívoros. Com isso, maior será o tempo necessário para a decomposição do material vegetal, retardando, assim, a liberação de nutrientes para o solo (GONZÁLEZ; SEASTEDT, 2001; STRICKLAND *et al*., 2009; MEWER; OSTERTAG; COWIE, 2011; RIUTTA *et al*., 2012; COLLISON; RIUTTA; SLADE, 2013; GARCIA-PALACIOS *et al*., 2013; WANG; YIN; LI, 2015). Sendo assim, a escolha do alimento torna-se uma questão relevante, pois tanto a qualidade quanto o tipo de recurso alimentar a ser consumido irão influenciar diretamente nos parâmetros populacionais dos consumidores (RUSHTON; HASSALL, 1983).

Elevadas concentrações de derivados fenólicos na serapilheira afetam negativamente o crescimento (ganho de massa) de isópodos terrestres (KOTILAINEN et al., 2009). Esse fato justifica a preferência por folhas em estágio avançado de decomposição, material vegetal mais palatável para oniscídeos (HASSALL; TURNER; RANDS, 1987; ZIMMER; KAUTZ; TOPP, 2003; SLADEA; RIUTTA, 2012), que, mesmo com menor quantidade de nutrientes, tem baixa concentração de fenólicos e baixa rigidez das folhas (HARBORNE, 1993; ZIMMER *et al*., 2002a; ZIMMER; KAUTZ; TOPP, 2003), tornando mais fácil o consumo.

1.4 Espécies do estudo

Nas duas sessões seguintes, serão abordados assuntos gerais referentes as duas espécies modelo utilizadas no presente estudo.

1.4.1 Carya illinoinensis

Carya illinoinensis (Wangenh) K. Koch (Juglandaceae) conhecida popularmente como nogueira pecan é uma espécie nativa da América do Norte, mas com distribuição atual em diversos locais, como na Europa, África e Ásia (HALL, 2000), e em países da América do Sul (LIM, 2011). No Brasil teve sua utilização inicial para fins econômicos em 1970, mas atualmente ocorre nos estados do Rio Grande do Sul, Santa Catarina e Paraná (RASEIRA, 1990). No Rio Grande do Sul a grande expansão da espécie foi favorecida pela comercialização da noz e da madeira (GATTO *et al*., 2008; BOSCARDIN; COSTA, 2018).

A nogueira é uma planta decídua, monoica e que pode chegar a aproximadamente 45 m de altura. As folhas são alternas, imparipinadas e lanceoladas (LIM, 2011). A espécie é citada pela alta produção de metabólitos secundários, como fenólicos, flavonoides (rutina, quercetina e outros) e esteroides (RYAN *et al*., 2006). Ácido gálico, rutina, quercetina, catequina, epicatequina e ácido elágico são derivados fenólicos amplamente encontrados na espécie (VILLARREAL-LOZOYA; LOMBARDINI; CISNEROS-ZEVALLOS, 2007; TREVISAN *et al*., 2014; HAWARY *et al*., 2015; BOTTARI *et al*., 2017; LEI *et al*., 2018). A noz também é reconhecida como uma excelente fonte de flavonoides, particularmente os monômeros flavan-3-ol e seus polímeros, além de taninos condensados (GU *et al*., 2004). A alta concentração de fenólicos encontrados em extratos da noz e da casca apresentam ação fungicida e antimicrobiana. O consumo da noz apresenta benefícios para a saúde humana, como ação antioxidante, prevenção de doenças cardiovasculares, diabetes, Alziemer e Parkinson (FRASER *et al*., 1992; LOMBARDINI; WALICZEK; ZAJICEK, 2008; ATANASOV *et al*., 2018). Em um recente estudo realizado por Bottari *et al*. (2017), foi verificado que extrato aquoso proveniente de folhas de nogueira foi capaz de inibir 20 espécies de microrganismos (bactérias e fungos). Os autores atribuíram este efeito inibitório aos derivados fenólicos encontrados na folha (ácido gálico, rutina, catequina, epicatequina e ácido elágico).

1.4.2 *Porcellio scaber*

O isópodo terrestre *Porcellio scaber* Latreille, 1804 é um importante detritívoro que compõem a comunidade edáfica com distribuição sinantrópica (NAIR, 1998). Na Europa Central é abundante em locais úmidos com muito material em decomposição (KOLAR *et al*., 2010). No Brasil é encontrado nos estados de Santa Catarina e Rio Grande do Sul (CAMPOS-FILHO; CARDOSO; AGUIAR, 2018). Apresentam um comportamento bem ativo durante a noite e nas primeiras horas do dia (NAIR, 1998). *Porcellio scaber* é utilizado amplamente em estudos devido ao seu tamanho, fácil amostragem, manipulação e cultivo em laboratório (DROBNE, 1997; STANEK; DROBNE; TREBSE, 2006; UDOVIC; DROBNE; LESTAN, 2009; GODET *et al*., 2012). A espécie tem sido muito utilizada na região Paleártica em estudos de bioindicação de ambiente, em virtude da capacidade dos organismos em acumular concentrações altas de metais pesados (HOPKIN, 1986; DROBNE, 1997; UDOVIC; DROBNE; LESTAN, 2009; GODET *et al*., 2011, 2012; GOSPODAREK et al., 2019; ZIDAR *et al*., 2019). Ganham destaque também em estudos de avaliação de impacto ambiental, principalmente em relação ao efeito de pesticidas (visto que não são as espécies-alvo) evidenciados em seus parâmetros populacionais (crescimento, taxas alimentares, sobrevivência, ganho de massa/peso, etc.) (DROBNE *et al*., 2008; STANEK; DROBNE; TREBSE, 2006), indicando, assim, ser um organismo chave do ambiente e bioindicador.

Em relação ao comportamento alimentar desta espécie, diversos estudos são realizados na Europa. Zimmer, Kautz e Topp (2005) mostraram que quando *P. scaber* consumiu folhas de *Quercus ruber* (L.) e *Alnus glutinosa* (L.) Gaertner, o isópodo aumentou a degradação de massa foliar e a disponibilização de restos vegetais no solo, favorecendo a atividade microbiana e liberação de nutrientes para o solo. Entretanto, Zimmer (1997) mostrou que, ao consumir alimento com alto teor de fenólicos (ácido gálico e ácido tânico), a tensão superficial do fluido intestinal de *P. scaber* aumentou. Desta forma, fica evidente que a qualidade do alimento influencia o consumo do isópodo (ZIMMER, 1999; ZIMMER; KAUTZ; TOPP, 2005, para mais detalhes).

Apesar de diversos estudos tratarem do efeito de derivados fenólicos (fenólicos totais e flavonoides totais) nas relações insetos-planta (MORIMOTO; KUMEDA; KOMAI, 2000; ONYILAGHA *et al*., 2004), pouco se sabe sobre o efeito destas substâncias nas relações detritívoro-planta. Considerando que *C. illinoinensis* possui alta quantidade de derivados fenólicos (fenólicos totais e flavonoides totais) e que recentemente foi observado o consumo de folhas da espécie por isópodos da espécie *P. scaber* (Soares, comunicação pessoal), é importante entender como ocorre está relação detritívoro-planta; bem como a variação dos derivados fenólicos (fenólicos totais e flavonoides totais) durante a decomposição foliar de *C. illinoinensis* e o efeito desta variação no comportamento alimentar do detritívoro *P. scaber*. Ao avaliar o efeito no comportamento alimentar de isópodos terrestres, poderemos evidenciar em nível individual, uma resposta com potenciais consequências ecológicas. Mesmo que *P. scaber* seja uma espécie alvo de muitos estudos na Europa, no Brasil há escassez de referências sobre o comportamento alimentar desta espécie em relação à sinalização química e ao conteúdo de fenólicos e flavonoides de espécies vegetais utilizadas como itens alimentares. Estudos com este enfoque podem evidenciar o papel de detritívoros na disponibilização de derivados fenólicos no solo. Além disso, considerando que *C. illinoinensis* possui alta quantidade dos flavonoides rutina e quercetina, os quais afetam diversos organismos, oferecer estes flavonoides em alimento artificial de maneira isolada, ou seja, sem a influência de outras substâncias encontradas nas folhas, pode evidenciar o real efeito de rutina e quercetina no comportamento dos isópodos terrestres.

Desta forma, o presente estudo visa caracterizar o comportamento alimentar de *P. scaber* frente a diferentes estágios de decomposição foliar de *C. illinoinensis*, bem como observar a influência isolada de dois flavonoides em um alimento artificial. O conhecimento acerca desses fatores evidenciará o momento no tempo em que os oniscídeos atuarão na matéria orgânica vegetal no solo. Esse comportamento tem grande relevância em diversos processos ecológicos, tais como ciclagem de nutrientes, transformação física da matéria orgânica de espaços naturais e facilitação da colonização por outros indivíduos.

1.5 Objetivo e estrutura da dissertação

Esta dissertação teve como objetivos verificar a influência do teor de fenólicos totais e flavonoides totais, presentes em diferentes estágios de decomposição foliar de *C. illinoinensis*, no comportamento alimentar de *P. scaber* (taxas de consumo, assimilação e egestão), além de investigar a influência de distintas concentrações de dois flavonoides (rutina e quercetina), oferecidos em alimento artificial, no comportamento alimentar de *P. scaber*.

A dissertação é composta por um artigo, o qual descreve a execução de dois experimentos: um primeiro oferecendo alimento natural (folhas de diferentes estágios de decomposição de *C. illinoinensis*) aos animais e outro com alimento artificial (gel de agarose) com diferentes concentrações de rutina e quercetina. No estudo foi verificada a quantidade de fenol total e flavonoides totais das folhas verdes de *C. illinoinensis* e também em folhas de um, dois, e três meses de decomposição em condições de campo. Os resultados dos dois experimentos foram estruturados através de comparações das taxas de consumo, taxas de assimilação e taxas de egestão entre os diferentes períodos de decomposição foliar e entre as distintas concentrações de ambos os flavonoides no alimento artificial. A discussão foi baseada na literatura proposta para o comportamento alimentar de *P. scaber* da região Paleártica em relação à quantidade e variação de derivados fenólicos durante a decomposição, bem como o que se tem registro para outras espécies de isópodos terrestres da região Neotropical e para outros grupos de fauna de artrópodos de solo. Nas considerações finais são apresentadas as conclusões do trabalho, relacionando os resultados obtidos com sugestões para estudos futuros.

Capítulo 1

Effect of Foliar Phenolic Compounds of *Carya illinoinensis* on the Feeding Behavior of *Porcellio scaber* (Isopoda: Oniscidea)[1]

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2 CAPÍTULO 1: Effect of Foliar Phenolic Compounds of *Carya illinoinensis* on the Feeding Behavior of *Porcellio scaber* (Isopoda: Oniscidea)

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Abstract – The study aims to verify whether the total phenolic content and total flavonoid content of the different decomposition stages of *Carya illinoinensis* leaves influences the feeding behavior (consumption, assimilation and egestion rates) of *Porcellio scaber*; and to evaluate the isolate effect of flavonoids rutin and quercetin, offered in artificial diet, in the feeding behavior of isopods. Green leaves of *C. illinoinensis* were put into litter bags and left in field for three months. Leaves were collected and offered monthly to the isopods in a no choice experiment. Total phenolic content and total flavonoid content in leaves were evaluated monthly. Agarose gel disks with flavonoids (0.0, 0.5, 1.0 and 2.0 mg / ml) were offered in a no choice experiment. The mean consumption rate and the mean egestion rate did not differ statistically among treatments (*Kruskal-Wallis*, H = 6.3059, P = 0.0976; H = 6.6857, P = 0.0826, respectively). Assimilation rate presented statistical difference. Three month old leaves differ from one month old and green leaves treatment (*Kruskal-Wallis*, H = 18.2856, P = 0.0004). Green leaves had the highest value of total phenolic content (185.441 ± 0.58 mg GAE/g dry weight) and total flavonoid content (209.057 ± 2.97 mg RE/g dry weight). Rutin and quercetin induced the highest mortality at 2.0 mg / ml concentration. It was not possible to identify an optimum concentration that stimulated feeding behavior of *P. scaber*. The feeding behavior of *P. scaber* was not inhibited by the presence of high total phenolic content and total flavonoid in the *C. illinoinensis* leaves.

Key-Words – Feeding rates, terrestrial isopods, Porcellionidae, decomposition, flavonoids.

INTRODUCTION

The gradual degradation process known as decomposition is very important for ecosystem functioning because it recycles nutrients and causes flow of mass through environments. This process is influenced by both abiotic and biological factors (Chew 1974; Attgnon et al. 2004). The chemical characteristics of the leaf litter, is important because impact the behavior of soil fauna and limit their consumption. Terrestrial isopods, a particular group of soil detritivores, contribute directly and indirectly to decomposition (Jia et al. 2015) and can accelerate this process (Sousa et al. 1998; Heneghan et al. 1999; Jia et al. 2015).

The production of secondary metabolites, such as phenolic derivatives (including flavonoids), has important adaptive functions in plants. Phenolic compounds act as epidermal filters (UV-A and UV-B protection) (Harborne 1993), inhibit fungal and bacteria growth (Osorio et al. 2010; Prado et al. 2014), besides, have antioxidant and enzyme inhibitory properties (Nwanna et al. 2019). Although these compounds are advantages to plants, they might be toxic to certain organisms. Isman (2002) showed that phenolic compounds act deterring feeding of desert locust (*Schistocerca gregaria* Forsk.), that is, consumption decreased in high concentrations.

Flavonoids, an important group of phenolic compounds, have also many functions in plants. They are responsible for flower coloration, pollinator signaling, protecting the plant from UV radiation and reducing the activity of both pathogens and herbivores (Adeyemi et al. 2010; Agati et al. 2011). These substances can intermediate insect-plant interactions as their deterrent properties can alter the behavior of herbivores, ultimately decreasing and/or inhibiting their plant consumption (Nwanna et al. 2019). Flavonoids can also be toxic to arthropods and can lead to their death (Gazzoni et al. 1997). Studies show that high concentrations of flavonoid quercetin (3,5,7,3’,4’-pentahydroxyflavone) inhibits the growth and behavior of insects, leading to decreased plant consumption (Adeyemi et al. 2010; Napal and Palacios 2015). When Lü and Liu (2016) offered different concentrations of flavonoid rutin (10, 30 and 90 g/m^2) to cigarette beetle *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), the behavior response was negatively affects and rutin repelled 100 % of beetles after 12 h exposure. Flavonoids can also influence other groups of organisms as showed by Jaisinghani (2017). The author found that flavonoid quercetin inhibited the growth of microorganisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*).

Terrestrial isopods are an important group for the environment, the presence of these detritivores is crucial to the decomposition process and might actually drive it in different environments (Sousa et al. 1998; Heneghan et al. 1999). Invertebrates fragment leaf litter as they consume it, increasing its surface area and facilitating the actions of other microorganisms (bacteria and fungi). They also disperse organisms present in feces to other sites and facilitate feces utilization by other soil fauna due increases in microbial biomass and extracellular enzyme activities (Jia et al. 2015). However, the chemical characteristics of leaf litter, such as phenolic compounds and lignin content, might inhibit and/or influence the feeding behavior (e.g. consumption) of terrestrial isopods, especially when in high content (Cameron and LaPoint 1978; Sousa et al. 1998; Heneghan et al. 1999; Wood et al. 2012; Gerlach et al. 2014; Faber et al. 2018). Consequently, the contribution of soil fauna to the decomposition process can be influence by the chemical characteristics of leaf litter (Zimmer 2002; Jia et al. 2015).

Soil fauna can perceive the chemical quality (Zimmer et al. 1996) and the different types of leaf litter in the environment (Quadros et al. 2014) through olfactory stimuli (Zimmer et al. 1996; Isman 2002). Consequently, this perception influences the behavior and feeding preferences of isopods (David et al. 2001; Loureiro et al. 2006). Although isopods feed on different litter types (Gerlach et al. 2014), woodlice exhibit a preference for softer litter with a low content of phenolic compounds, a low C:N ratio and a high N content (Pennings et al. 2000; Zimmer and Topp 2000; Zimmer 2002; Wood et al. 2012; Quadros et al. 2014). These characteristics make foliar material more palatable and therefore increase its consumption and preference (Pennings et al. 2000; Quadros et al. 2014; Faber et al. 2018). Boelter et al. (2009) showed that the neotropical isopod *Benthana cairensis* (Sokolowicz, Araujo and Boelter, 2008) prefers and consumes more decomposed leaves (three month old leaves). This can be due to the fact that degradation of phenolic compounds during decomposition increases the palatability of the leaf litter (Pennings et al. 2000). Recalcitrant litter (tough litter with a high phenolic content) is generally avoided by detritivores (Pennings et al. 2000), because it directly affects the population parameters of woodlice such as reproductive output, growth and survival (Sousa et al. 1998; Caseiro et al. 2000; Kautz and Zimmer 2000; Jia et al. 2015).

Carya illinoinensis (Wangenh.) C. Koch (Juglandaceae) is a native North-American hickory cultivated in Australia, South Africa, Israel and South America (Call et al. 2006; Prado et al. 2009a). The species is deciduous and monoecious, being about 20 to 30 meters high (40 meters when raised under good conditions). Leaves are alternate and pinnate, with nine to 15 leaflets (Fronza et al. 2018). Studying flavonol glycosides, Ishak et al. (1980) found quercetin 3-glucoside, quercetin 3-galactoside, quercetin 3-rhamnoside, quercetin 3-arabinoside and kaempferol 3-monomethyl in the leaves. Furthermore, this species has a high concentration of hydrolyzable tannins and flavonoid derivatives such as proanthocyanidins and flavonols in its nut shells and kernels with antimicrobial, bactericidal activities and antitumor activity (La Rosa et al. 2011; Prado et al. 2014; Hilbig et al. 2017). These substances present in kickory can influence organisms. Flores-Estrada et al. (2019) found that nutshell extract of *C. illinoinensis* (two varieties) inhibited the activity on *S. aureus,* due to the presence of phenolic derivatives.

Porcellio scaber Latreille, 1804 is an important detritivore that contribute to fragmentation of leaf litter. This species contributes to nutrient cycling in environments (Lapanje et al. 2007). Kautz and Topp (2000) showed that *P. scaber* increased the leaf litter degradation of *Quercus ruber* (L.) e *Alnus glutinosa* (L.) Gaertner. However, the fragmentation and nutrient release of *A. glutinosa* (L.) occurred faster. Gerlach et al. (2014) showed that *P. scaber* preferred fed on litter originated from introduced tree species (*Quercus ilex*) than native species (*Q. robur*) while other isopods as *Oniscus asellus* (Linnaeus, 1758) showed preference for native species and avoided *Q. robur*.

Recently, *P. scaber* was encountered feeding on *C. illinoinensis* leaves (Soares, personal communication). Although studies relate the effect of secondary metabolites to the behavior of arthropods, and this is commonly investigated for insects (Zimmer et al. 1996), it is scarcely investigated in relation to terrestrial isopods. Furthermore, considering that litter with high content of phenolic derivatives (including flavonoids) is normally avoided or less consumed by woodlice species, and that *C. illinoinensis* contains high quality and quantity of phenolic derivatives that negatively affect many other organisms, it is important to study the detritivore-plant relationship between these two exotic species to understand the effect of this interaction in behavior of *P. scaber.* Furthermore, it is important to study the dynamics of phenolic derivatives (total phenolic content and total flavonoid content) during the decomposition process of *C. illinoinensis* leaves, as well as the possible effect of this dynamic on the feeding behavior of detritivore *P. scaber*. Studying the effect on feeding behavior of isopod, can evidence, in organism level, a response that has potential ecological consequences. According Ihnen and Zimmer (2008) is important to have studies which focus on the metabolites involved in plant-isopod interactions, because these compounds largely influence the feeding rates and behaviors of isopods. Besides, as *C. illinoinensis* has a great amount of flavonoids (rutin and quercetin) that negatively affect herbivores, and little is known about its effect on isopods, is important to offer them in isolation food (without other compounds found in leaves) to elucidate the real effect of these two compounds on the feeding behavior of terrestrial isopods.

For this reason, the present study aims to verify whether the total phenolic content and total flavonoid content of the different decomposition stages of *C. illinoinensis* leaves influence the feeding behavior of *P. scaber* (consumption rate, assimilation rate and egestion rate); and to evaluate the effect of different concentrations of two flavonoids (rutin and quercetin) in the feeding behavior of isopod. This study will also determine if there is an optimal concentration of these compounds that was stimulates isopod feeding behavior (increases the consumption). The hypotheses are: 1 – During the decomposition process of *C. illinoinensis* the content of the phenolic derivatives will gradually decrease; 2 – Low phenolic and flavonoid content in the late stages of litter decomposition will stimulate the feeding behavior of *P. scaber*; 3 – The gradual increase of the concentration of flavonoids (rutin and quercetin) in artificial foods will reduce the feeding rates of *P. scaber*.

METHODS AND MATERIALS

Species Source Sites Leaves of *C. illinoinensis* were collected from three specimens in Porto Alegre (Parque Moinhos de Vento), state of Rio Grande do Sul (30º 01’38.6” S, 51º 12’05.8” W), in march 2018. The foliar material was taken to the Laboratório de Ecologia Química e Quimiotaxonomia (LEQTAX) of the Universidade Federal do Rio Grande do Sul (UFRGS) and put into several litter bags (20 x 30 cm, mesh size 1.0 mm) (Crossley and Hoglund 1962) and left in field conditions (see below) for one (T1), two (T2) or three month (T3) (from March to June) for use in the feeding behavior experiments and for analysis of its phenolic derivatives (total phenolics and total flavonoids). On the day of collection part of the material (additional green leaves that were not placed into the *litter bags*) was taken also for use in the feeding behavior experiments and for analysis of its phenolic compounds (total phenolics and total flavonoids). This sample of green leaves constituted time zero (T0) that is the starting point for the analysis of the present study.

The leaves than left in field conditions, were placed side by side inside the litter bags to undergo the same decomposition process. The litter bags were placed in field conditions under the forest floor at Ufrgs Campus do Vale (30º 03’58.0” S, 51º 07’21.7” W) (in the other site where leaves were collected from) (Fig. 1). The vegetation of local is identified by Brack et al. (1998) as high forest community, with 12 - 20 m high and presence of three or four tree strata, and characterized as Estacional Semidecidual Forest (Ibge 2012). During the period in which litter bags stayed in that place (from March to June), rainfall (precipitation in millimeters) was verified (Cptec-Inpe 2018) (see Annex for more details). The amount of precipitation in the local was 100.1 to 200.0 mm for March and April, 50.0 to 100.0 mm for May and 150.0 to 200.0 mm for June. These data were used because rainfall occurred during the period when the litter bags were left in the field, and also because it always rained on the days prior to litter bag collection and might influence the results.

Porcellio scaber individuals were collected monthly from urban area in Caxias do Sul, state of Rio Grande do Sul (29º 09’09.0” S 51º 10’30.9” W). They were transported to the laboratory (LEQTAX) where they were maintained in translucent plastic boxes containing *C. illinoinensis* leaves and soil substrate, both obtained from the source area and maintained in laboratory at 20 ± 1°C and under 12:12h (light:dark) photoperiod.

Fig. 1 Litter bags in field conditions.

Evaluation of P. scaber Feeding Behavior on Different Decomposition Stages of Carya illinoinensis Leaves The experimental units (90 x 10 mm petri dishes) contained one individual *P. scaber*, one leaf disk and filter paper in the bottom to maintain the internal humidity (0.5 ml distilled water was added every two days or when necessary). Although the number of sampling units varied between treatments, a minimum of 12 sample units were maintained. Only adult *P. scaber* weighing between 30 to 45 mg were used. Both males and females were used, but ovigerous females were excluded. We use only *P. scaber* individuals that were in an intermoult stage during experiment.

Green leaves (T0) and leaf litter (at each decomposition stage in months T1, T2, T3) of *C. illinoinensis* were cut into disks with a diameter of 18 mm, oven dried at 40 ± 1°C for 48 hours, weighed and remoistened with distilled water before being offered to isopods in a no-choice experiment. The experiment lasted eight days or until 80 % of the disks had been consumed. The digestive tract of tested animals was emptied at the beginning and end of the experiment by feeding them only with carrot for two days (Wood et al. 2012). This ensured that only egested leaves of *C. illinoinensis* were collected during the experiment.

The treatments consisted of disks of green leaves, one month old, two month old or three month old leaves (T0, T1, T2 and T3 respectively), representing four decomposition stages. Control units represented autogenic loss (natural mass loss of leaves in the absence of isopods) Szlávecz (1985). Feces were collected every day to minimize coprophagy and were armazened at -20°C for feeding behavior analyses. After the experiment the isopods, their feces and the remaining leaf disks were oven dried and weighed to calculi feeding rates.

The mean of feeding rates (consumption rate, assimilation rate and egestion rate) were calculated according to Wood et al. (2012) (using mg instead of g) adapting Loureiro et al. (2006):

(1) $CR = [(m_{if} – m_{ff}) – m_a] / m_{isop} * day$

(2) $AR = \{[(m_{if} – m_{ff}) – m_a] - m_f\} / m_{isop} * day$

(3) $ER = m_f / m_{dwi} * day$

Where: (1) CR = Consumption rate; m_{if} = initial food mass (DW); m_{ff} = final food mass (DW); m_a = mean percentage of mass lost in autogenic units (DW); m_{isop} = mean isopod mass (FW); (2) AR = Assimilation rate; m_f = mass of feces (DW); (3) ER = Egestion rate; m_{dwi} = mean isopod mass (DW) (DW = dry weight; FW = fresh weight). The total mortality per treatment were recorded and given as a percentage (%).

Evaluation of the Total Phenolic and Total Flavonoid Content of Carya illinoinensis Leaves Throughout its Decomposition Process

Preparation of the C. illinoinensis Extract The aqueous extracts of *C. illinoinensis* were prepared with green leaves (T0) or leaf litter from each decomposition stage; one month old, two month old and three month old leaves (T1, T2 and T3 respectively).

Leaves (10 g) were oven-dried at 50°C for 24 hours. The aqueous extract was warmed in 100 ml of distilled water at 90°C for 10 minutes. Next, the volume was adjusted to 100 ml and maintained at 20 ± 1°C for one day. Finally, the extract was filtered with filter paper.

Determination of Total Phenolic Content The total phenolic content was analyzed with a spectrophotometer by adapting the Folin-Ciocalteu colorimetric method (Prado et al. 2009b). A detailed version can be seen in Waterman and Mole (1994). An aliquot of aqueous extract (100 μl) and 500 μl of Folin-Ciocalteu reagent were added to a 10 ml volumetric flask and mixed. After 5 minutes 1.5 ml of Na_2CO_3 (10 g in 50 ml of distilled water) was added, mixed and the total volume was made up to 10 ml with distilled water. The entire procedure was performed in a dark room. Samples were analyzed in triplicate. Sample absorbance was determined at 764 nm with a Micronal B582 spectrophotometer two hours after the addition of Na_2CO_3. Gallic acid was used to calibrate the equipment (concentrations: 0.2, 0.25, 0.30, 0.35, 0.4, 0.5, 0.6, 0.7, 0.75 and 0.8 mg/l of distilled water). Absorbance values were plotted to obtain a trend line and the equation of this line (Fig. 2). The equation of the trend line was used to calculate milligrams of gallic acid equivalents per ml of extract (mg GAE/ml). This value was converted into total phenolic content and was expressed as mg of gallic acid equivalents per g of leaf dry weight (mg GAE/g dry weight).

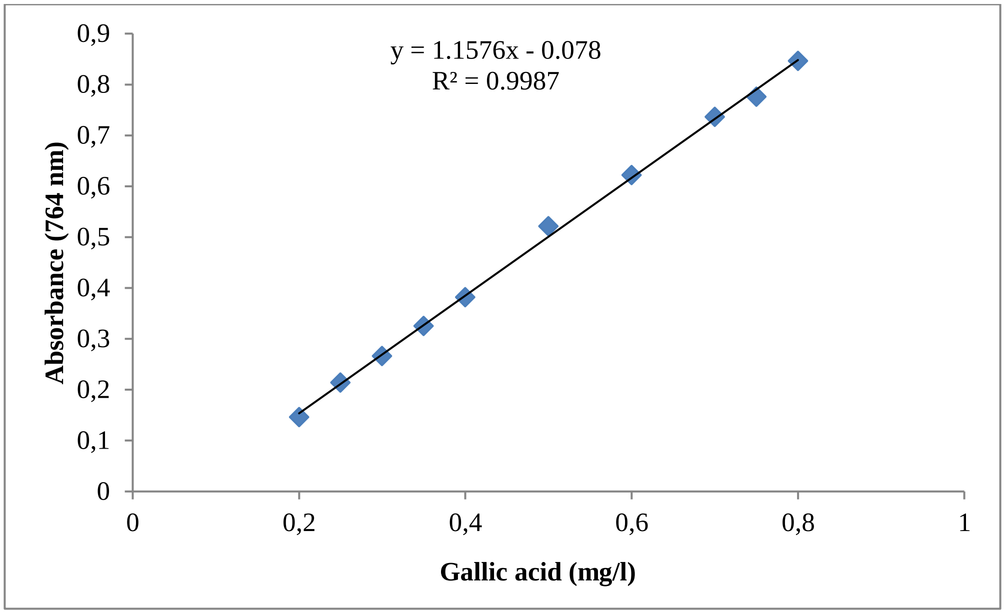

Fig. 2 Gallic acid calibration curve.

Determination of Total Flavonoid Content The total flavonoid content was analyzed with a spectrophotometer by adapting the aluminum chloride colorimetric method (Marinova et al. 2005). An aliquot of distilled water (4 ml) was added to a 10 ml volumetric flask and mixed with 1 ml of extract before 0.3 ml of $NaNO_2$ 5% was added. After 5 minutes 0.3 ml of $AlCl_3$ 10 % was added and mixed. Six minutes later 2 ml of NaOH 1M was added and the volume was made up to 10 ml with distilled water. The procedure was completed in a dark room and all samples were analyzed in triplicate. The solution was mixed and the absorbance was determined at 510 nm with a Micronal B582 spectrophotometer. Rutin was used to produce the calibration curve (concentrations: 0.15, 0.2, 0.25, 0.30, 0.35, 0.4, 0.45, 0.5, 0.55 and 0.6 mg/l of distilled water). Sample absorbance were plotted to obtain a trend line and the equation of this line (Fig. 3). The equation was used to calculate milligrams of rutin equivalents per ml of extract (mg RE/ml). This value was converted into the total flavonoid content, expressed as mg of rutin equivalents per g of leaf dry weight (mg RE/g dry weight).

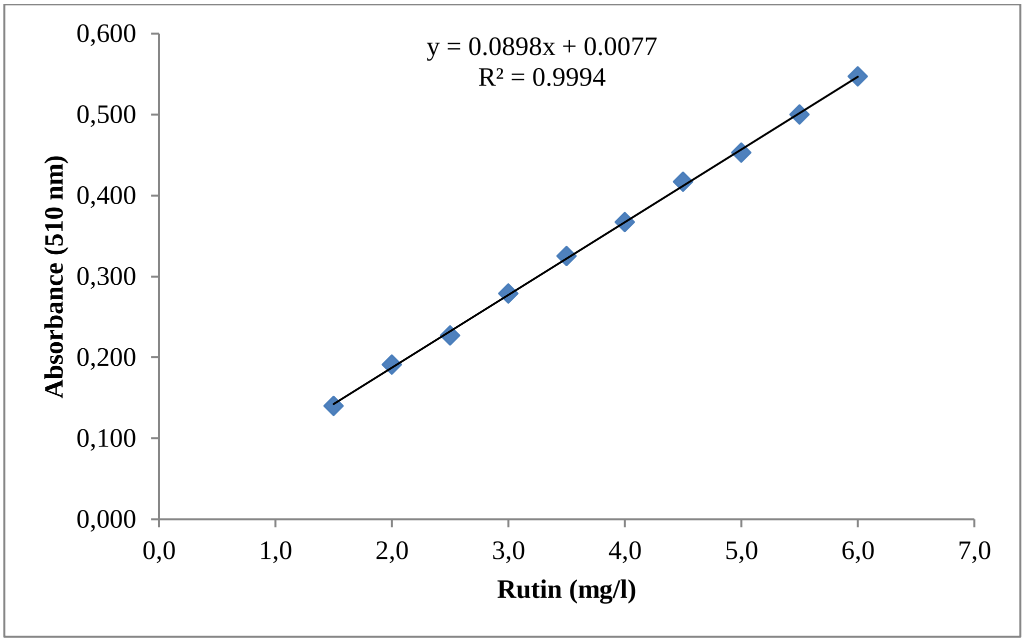

Fig. 3 Rutin calibration curve.

To determine the total amount of phenol and flavonoid ingested by isopods (mg) the means of the two metabolites were multiplied by the consumption rates.

Preparation of Quercetin and Rutin gels Agarose (380 mg) was dissolved in 27 ml of distilled water at 90°C. The solution was cooled at 60°C and mixed with a stock solution of quercetin (10 mg/ml). Different concentrations were obtained after addition of ethanol PA (0.0, 0.5, 1.0 and 2.0 mg/ml). The mixture of agarose and quercetin was placed in Petri disks and kept at 20 ± 1°C overnight to cool and to gel (Kenne, unpublished data). The same procedure was performed for rutin. The different concentrations were chosen based on the evaluation of the total phenolic and total flavonoid content of *C. illinoinensis* leaves.

Effect of Rutin and Quercetin on the Feeding Behavior of Porcellio scaber The experimental units (90x100 mm petri dishes) consisted of an individual *P. scaber*, one artificial food disk and filter paper covering the bottom of the disk. This filter paper maintained the internal humidity (0.5ml of distilled water was applied every two days or when necessary). Although the number of sampling units varied between treatments, a minimum of 6 sample units were maintained. Only adults' *P. scaber* with body weights ranging between 30 to 45 mg were used. Both males and females were used but ovigerous females were excluded. We use only *P. scaber* individuals that were in an intermoult stage during experiment.

The agarose gels were cut into 18 mm-diameter disks, weighed and offered as food to isopods over five days in no-choice experiment. Isopods were only given carrot as a food source for two days at the beginning and end of the experiment, so that only egested leaves which were consumed during the experiment were collected (Wood et al. 2012). The units were kept at 20 ± 1°C under 12:12 (light:dark) photoperiod. Treatments consisted of quercetin or rutin gel disks with different concentrations described above. Feces were collected every day and armazened at -20°C to allow the calculation of feeding rates. After the experiment the isopods and collected feces were oven dried at 60 ± 1°C for 48 hours before being weighed. The disks of agarose gel were also weighted at the end of the experiment. Feeding rates were calculated as see above. The total mortality per treatment were recorded and given as a percentage (%).

Statistical Analyses The *Kolmogorov-Smirnov test* was used to verify that the data had a normal distribution. When the data had a normal distribution a *one-way analysis* of variance (ANOVA) was used to compare the mean consumption, egestion and assimilation rates of *P. scaber* fed with leaves, agarose-rutin or agarose-quercetin. Following this *pos-hoc Tukey's test* was used to evidence the statistical differences between the treatments. When data was found to not have a normal distribution, a *Kruskal-Wallis test* was used to compare the data and *Dunn's test* was used when necessary to highlight the significant differences. In order to verify the relationship between the consumption and egestion rates among treatments the Pearson correlation index was used. All statistical analyses were run in BioEstat 5.0 or IBM SPSS Statistics 22.

RESULTS

Evaluation of P. scaber Feeding Behavior on Different Decomposition Stages of Carya illinoinensis leaves The mean consumption rate of isopods were not statistically different when feeding on the different decomposition stages of *C. illinoinensis* (*Kruskal-Wallis*, H = 6.3059, P = 0.0976). However, a slight tendency of decrease in the mean consumption rate from green leaves (1.37 mg/mg^{-1} day^{-1}) to two month old leaves, and a slight tendency of increase from two month old leaves to three month old leaves (1.40 mg/mg^{-1} day^{-1}) were observed (Fig. 4). No statistical differences were found among the isopods' mean egestion rate (*Kruskal-Wallis*, H = 6.6857, P = 0.0826). However, the mean egestion rate shows a tendency decrease accompanying the age of the leaves, from green leaves to two month old leaves, and a tendency of increase from two month old leaves to three month old leaves (5.56 mg/mg^{-1} day^{-1}) (Fig. 5). The mean assimilation rate of three month old leaves differed from the assimilation rate of green leaves and one month old leaves, but not from that of two month old leaves (*Kruskal-Wallis*, H = 18.2856, P = 0.0004) (Fig. 6).

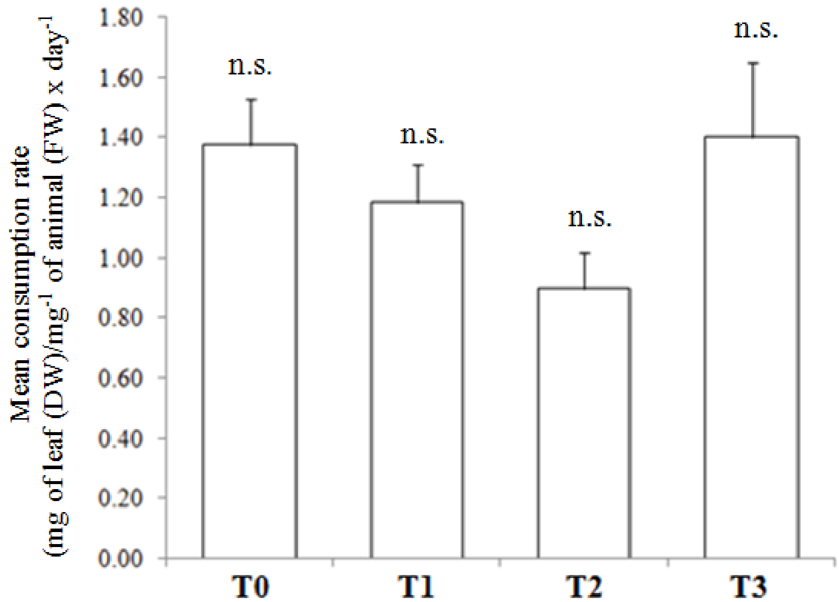

Fig. 4 Mean consumption rate of *Porcellio scaber* fed on leaf disks of *Carya illinoinensis* in a no-choice experiment lasting 8 days. Each treatment corresponded to a decomposition stage (T0 = green leaves; T1 = one month old leaves; T2 = two month old leaves and; T3 = three month old leaves). Vertical bars indicate the standard error. n.s. represents the absence of significant differences. Sample number (N = 17; 12; 17; 15 for T0, T1, T2 and T3 respectively).

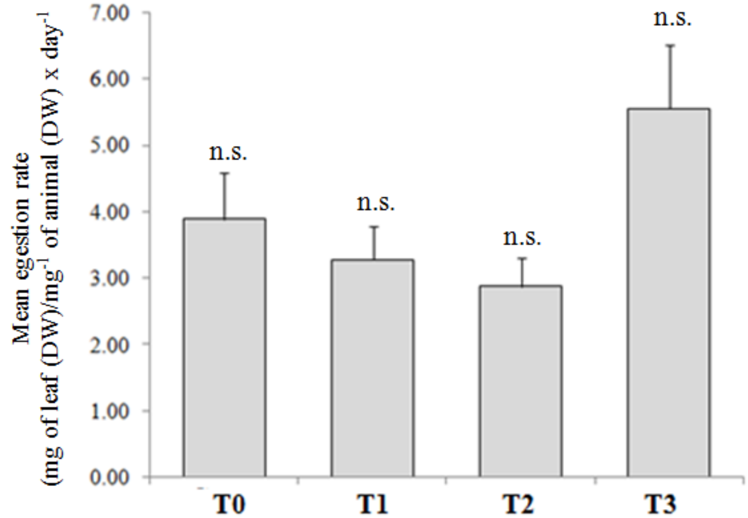

Fig. 5 Mean egestion rate of *Porcellio scaber* fed on leaf disks of *Carya illinoinensis* in a no-choice experiment lasting 8 days. Each treatment corresponded to a decomposition stage (T0 = green leaves; T1 = one month old leaves; T2 = two month old leaves and; T3 = three month old leaves). Vertical bars indicate the standard error. n.s. represents the absence of significant differences. Sample number (N = 17; 12; 17; 15 for T0, T1, T2 and T3 respectively).

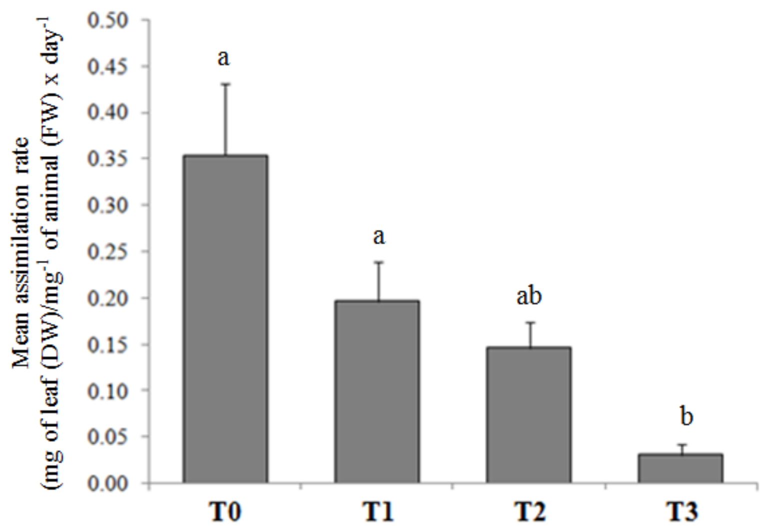

Fig. 6 Mean assimilation rate of *Porcellio scaber* fed on leaf disks of *Carya illinoinensis* in a no-choice experiment lasting 8 days. Each treatment corresponded to a decomposition stage (T0 = green leaves; T1 = one month old leaves; T2 = two month old leaves and; T3 = three month old leaves). Vertical bars indicate the standard error. Different letters above the bars represent a statistical difference ($\alpha<0.05$) while the same letters represent no statistical difference. Sample number (N = 17; 12; 17; 15 for T0, T1, T2 and T3

All decomposition stages showed statistically different correlations between consumption and egestion rate. The three month old leaves had the strongest correlation ($R^2 = 0.9679$, $P < 0.0001$) followed by the two month old leaves ($R^2 = 0.9510$, $P < 0.0001$), the one month old leaves ($R^2 = 0.9490$, $P < 0.0001$) and lastly the green leaves ($R^2 = 0.7789$, $P = 0.0002$) (Table 1).

Total mortality of isopods (%) was high to T3 (26.32 %) and was higher than in T0 (16 %). No mortality was recorded in the treatment using T1 (Fig. 7).

Table 1 Pearson correlation of the consumption and egestion rates of *Porcellio scaber* fed on leaf disks of *Carya illinoinensis* in a no-choice experiment lasting 8 days. Each treatment corresponded to a decomposition stage.

Treatments	R^2	*P*
Green leaves (T0)	0.7789	0.0002
One month old leaves (T1)	0.9490	<0.0001
Two month old leaves (T2)	0.9510	<0.0001
Three month old leaves (T3)	0.9679	<0.0001

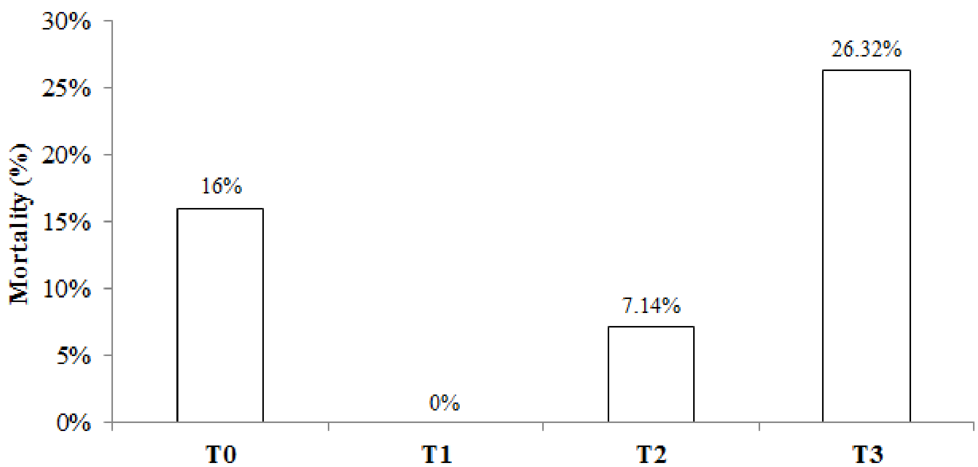

Fig. 7 Total mortality (%) of *Porcellio scaber* fed on leaf disks of *Carya illinoinensis* for 8 days of experiment. Each treatment corresponded to a decomposition stage (T0 = green leaves; T1 = one month old leaves; T2 = two month old leaves and; T3 = three month old leaves). Total mortality is given as a percentage (%). Sample number (N = 17; 12; 17; 15 for T0, T1, T2 and T3 respectively).

Evaluation of the Total Phenolic and Total Flavonoid Content of Carya illinoinensis Leaves Throughout its Decomposition Process The total phenolic content differed significantly among all evaluated decomposition stages (*Kruskal-Wallis*, H = 9.6525, P = 0.0218). Green leaves (T0 treatment) had the highest value of phenolic content (185.441 ± 0.58 mg GAE/g dry weight). The value obtained in the T0 treatment was four times higher than T1 and T2 (approximately). Three month old leaves (T3 treatment) had the lowest phenolic content (36.512 ± 0.12 mg GAE/g dry weight), value five times lower than T0 (approximately). Although the total phenolic content decreased according to the decomposition stage, the only significant difference was found between green leaves and three month old leaves (Table 2).

Total flavonoid content differed significantly among all evaluated decomposition stages (*Kruskal-Wallis*, H = 10.3846, P = 0.0156). Green leaves (T0 treatment) had the highest flavonoid content (209.057 ± 2.97 mg RE/g dry weight). T0 treatment was six times higher than T1 and 80 times higher than T3 (approximately). The lowest flavonoid content was recorded in two month old leaves (0 ± 0.00 mg RE/g dry weight), a value 200 times lower than T0 (approximately). Total flavonoid content decreased as decomposition stage increased, except for the three month old leaves which had slightly higher flavonoid content than the two month old leaves. The only significant difference was found between green leaves and two month old leaves (Table 2).

Table 2 Total phenolic content in mg of gallic acid equivalents per g of leaf dry weight (DW) and total flavonoid content in mg of rutin equivalents per g of leaf dry weight (DW) of *Carya illinoinensis* leaves. Values correspond to the mean ± SE. Different letters represent statistically different values (α<0.05).

Treatments	Phenolic content (mg eq. GAE/g DW)	Flavonoid content (mg eq. RE/g DW)
Green leaves (T0)	185.441 ± 0.58^{a}	209.057 ± 2.97^{a}
One month old leaves (T1)	42.156 ± 0.20^{ab}	31.644 ± 1.34^{ab}
Two month old leaves (T2)	39.046 ± 1.74^{ab}	0 ± 0.00^{b}
Three month old leaves (T3)	36.512 ± 0.12^{b}	2.450 ± 1.06^{ab}

A significantly larger amount of phenolics and flavonoids was ingested by *P. scaber* when they consumed green leaves than from leaves at other stages of decomposition (*Kruskal-Wallis*, H = 34.6895, P < 0.0001) (Fig. 8). There were no significant differences in the ingestion of the phenolic content among these other decomposition stages. However, there was more variation among the ingestion rates of flavonoids (*Kruskal-Wallis*, H = 55.9772, P < 0.0001) (Fig. 8). There were no differences in the ingestion between green leaves and one month old leaves or between one month old leaves and three month old leaves. However, the ingestion of phenolics and flavonoids in two month old leaves differ from that of all other decomposition stages.

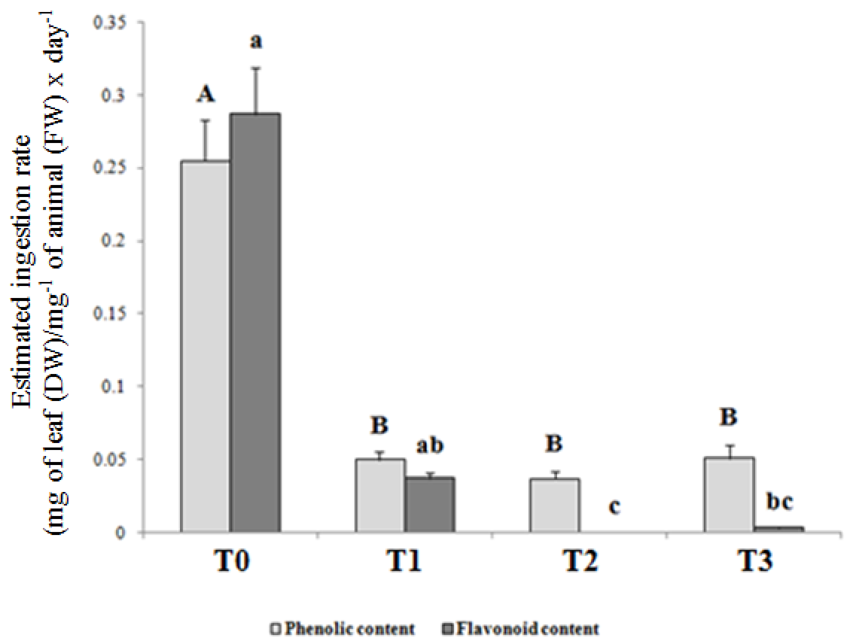

Fig. 8 Estimated ingestion rate of derivate phenolics and flavonoids compounds by *Porcellio scaber* individuals fed on leaf disks of *Carya illinoinensis* at different treatments in an 8 days of experiment. Each treatment corresponded to a decomposition stage (T0 = green leaves; T1 = one month old leaves; T2 = two month old leaves and; T3 = three month old leaves). Values correspond to the mean ± SE. Capital case letters compare treatment with phenolic content and lower case letters compare treatment with flavonoid content. Different upper case and lower case letters show statistical differences ($\alpha<0.05$) inside treatments. Data is given in mg/mg^{-1} of isopods per day^{-1}. Sample number (N = 17; 12; 17; 15 for T0, T1, T2 and T3 respectively).

Effect of Rutin and Quercetin on the Feeding Behavior of Porcellio scaber Agarose gel treatments containing different concentrations (0.0; 0.5; 1.0 and 2.0 mg/ml) of rutin produced no statistical differences among the mean consumption rate (*ANOVA*, F = 0.4237, P = 0.7409), mean assimilation rate (*ANOVA*, F = 0.4392, P = 0.7303) and mean egestion rate (*ANOVA*, F = 0.8018, P = 0.5067) of *P. scaber* (Table 3). However, a small increase in mean consumption rate and assimilation rate with increasing rutin concentration was observed, but there was a small decrease in mean egestion rate with increasing rutin concentration.

Table 3 Consumption, egestion and assimilation rates of *Porcellio scaber* fed on agarose containing different concentrations of rutin (0.0, 0.5, 1.0 or 2.0 mg/ml) for five days. Values of feeding rates correspond to the mean ± SE. Agarose gel weight (mg) per isopod weight (mg). Rutin sample number (N = 8; 8; 8; 6 for 0.0, 0.5, 1.0 and 2.0 mg/ml respectively).

Concentration (mg/ml)	Consumption (mg/mg*day)	Egestion (mg/mg*day)	Assimilation (mg/mg*day)
0	9.56 ± 2.11	0.36 ± 0.10	9.47 ± 2.10
0.5	11.15 ± 0.92	0.33 ± 0.09	11.07 ± 0.91
1.0	11.79 ± 1.85	0.27 ± 0.07	11.72 ± 1.85
2.0	11.85 ± 1.20	0.18 ± 0.06	11.81 ± 1.21

In the quercetin experiment, the mean egestion rate was the only metric that was statistically different regarding the egestion rate (*Kruskal-Wallis*, H = 11.2809, P = 0.0103). The difference was observed between the concentrations 2.0 mg/ml and 1.0 mg/ml as well as between the concentrations 2.0 mg/ml and 0.5 mg/ml. Control was not different from other treatments. No statistical difference was observed for mean consumption rate (*ANOVA*, F = 1.2303, P = 0.3140) and mean assimilation rate (*ANOVA*, F = 1.2110, P = 0.3209) (Table 4), however, there was a slightly decrease in mean consumption rate and mean assimilation rate from the concentration 1.0 mg/ml to 2.0 mg/ml. No significant correlation was found between mean consumption and mean egestion rates for both the rutin and quercetin treatments (Table 5).

Table 4 Consumption, egestion and assimilation rates of *Porcellio scaber* fed on agarose treatments containing different concentrations (0.0, 0.5, 1.0 or 2.0 mg/ml) of quercetin for five days. Values correspond to the mean ± SE. Different letters show statistical differences (α<0.05). Agarose gel weight (mg) per isopod weight (mg). Quercetin sample number (N = 11; 11; 8; 7 for 0.0, 0.5, 1.0 and 2.0 mg/ml respectively).

Concentration (mg/ml)	Consumption (mg/mg*day)	Egestion (mg/mg*day)	Assimilation (mg/mg*day)
0	12.82 ± 2.20	0.32 ± 0.05^{ab}	12.74 ± 2.20
0.5	9.43 ± 1.07	0.54 ± 0.12^{a}	9.3 ± 1.06
1.0	13.92 ± 1.44	0.94 ± 0.28^{a}	13.69 ± 1.44
2.0	11.17 ± 2.29	0.12 ± 0.03^{b}	11.14 ± 2.28

Table 5 Pearson correlation of the consumption and egestion rates of *Porcellio scaber* fed on artificial food containing different concentrations of Rutin and Quercetin for five days. Concentrations are given in mg/ml.

Flavonoid	**Rutin**		**Quercetin**	
Concentration (mg/ml)	R^2	P	R^2	P
0	0.3703	0.3665	0.3143	0.34
0.5	0.4803	0.2283	0.3473	0.2952
1.0	0.1448	0.7322	-0.044	0.9176
2.0	-0.3016	0.5613	0.6859	0.0888

In the rutin experiment, the total mortality of *P. scaber* only occurred in the highest concentration treatment (2.0 mg/ml) (Fig. 9), where 25 % of the isopods died. There was no mortality in the other treatments. In the quercetin experiment the total mortality of *P. scaber* increased with concentration (Fig. 10).

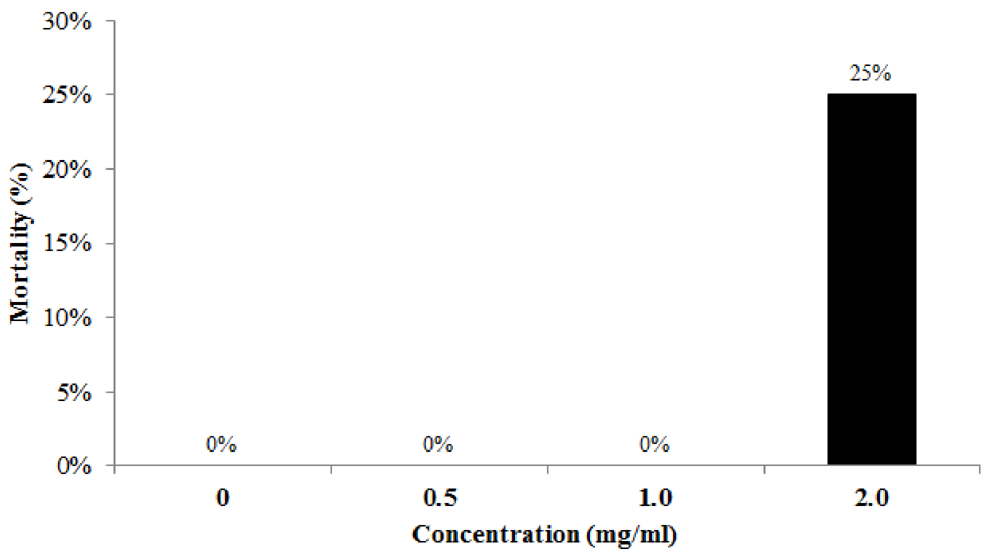

Fig. 9 Total mortality of *Porcellio scaber* fed on agarose containing different concentrations of rutin for five days. Concentrations are given in mg/ml. Rutin sample number (N = 8; 8; 8; 6 for 0.0, 0.5, 1.0 and 2.0 mg/ml respectively).

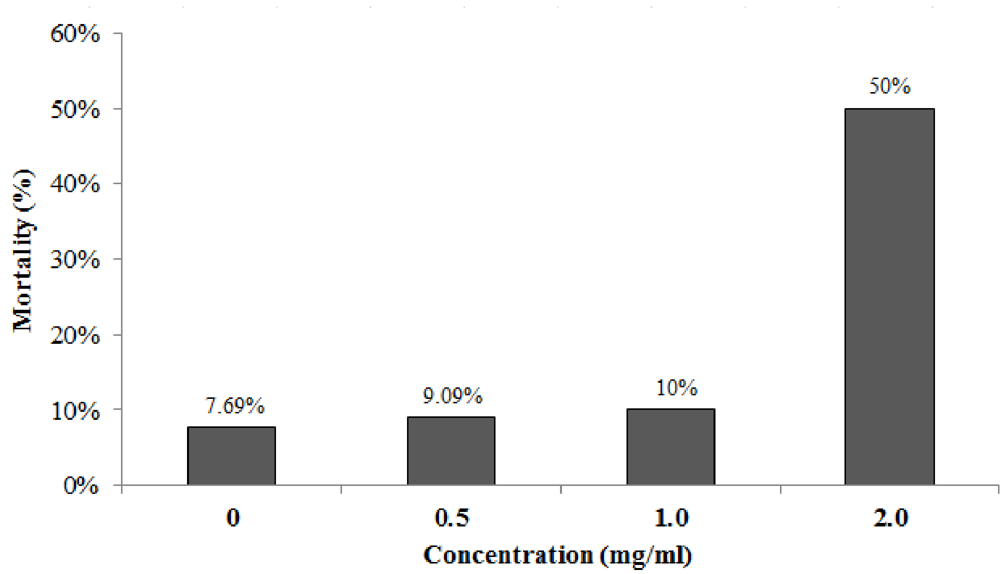

Fig. 10 Total mortality of *Porcellio scaber* fed on agarose containing different concentrations of quercetin for five days. Concentrations are given in mg/ml. Quercetin sample number (N = 11; 11; 8; 7 for 0.0, 0.5, 1.0 and 2.0 mg/ml respectively).

DISCUSSION

This study detected a high content of total phenolic compounds and total flavonoids in the green leaves of *C. illinoinensis* in an anthropized environment (Parque Moinhos de Vento). Hawary et al. (2015) who studied plant material collected from trees under cultivation conditions (monoculture) in Egypt, found approximately half of the total phenolic content (98.64 ± 0.68 mg GAE/g and 103.27 ± 0.33 mg RE/g). Studies have shown that a high phenolic content in green leaves functions as plant defense from several kinds of biotic and abiotic stress, such as herbivores and ultraviolet radiation (Harborne 1993; Pavia et al. 1997; War et al. 2012). Although studies in this field have been carried out in different places, monoculture relies widely on products and additional soil nutrients in order to reduce herbivory, pathogens and plant diseases. These factors might reduce plant defense investment in such systems, but the same are not present in urban environments. Therefore, absence of these factors (management) on trees in Parque Moinhos de Vento might contribute to plants' investment in defenses, which may explain the high quantity of phenolic content (total phenolic and total flavonoid content) in the green leaves analyzed in our study. Furthermore, this initial high phenolic derivatives content might be important to detritivores, yet food with high secondary metabolites might influence isopods behavior (see below).

In the present study, it was observed a decrease in the content of phenolic derivatives (total phenolic and total flavonoid content) with decomposition; this result confirms the first hypotheses, that during the decomposition process of *C. illinoinensis,* the content of the phenolic derivatives would gradually decrease. Chomel et al. (2014) found a decrease in phenolic content during the decomposition of *Pinus halepensis*. Although the authors studied a different tree species, they also found high content of phenolic derivatives in green leaves (needles) and a decrease of these compounds during the decomposition process (approximately 80 % after 6 months). Studying leaching of phenolic compounds, Kuiters and Sarink (1986) found similar results. The authors showed that the high phenolic content of the freshly fallen leaves of deciduous tree species was quickly leached, and, after four months of decomposition, a great amount of phenolic content had been leached from litter (*e.g. p*-hydroxy benzoic acid, vanillic acid, protocatechuic acid, gallic acid, gentisic acid, ferulic acid, *p*-coumaric acid and *o*-coumaric acid). Kuiters and Sarink (1986) attributed this rapid loss to the phenolic content's high water-solubility (low molecular weight). Studying nine species of nut tree, Yang et al. (2009) found that walnuts (*Juglans regia* L.) and pecan (*C. illinoinensis*) have higher soluble phenolic content than other species, and that pecan have a higher concentration of soluble flavonoids. Therefore, considering that *C. illinoinensis* is a deciduous species with a high amount of soluble secondary metabolites, the breakdown of leaf cells and release of phenolic derivatives (of low molecular weight), which happen rapidly during early decomposition stages, might have been strongly influenced by the rainfall during the period in which the litter bags were left in the field (from March to June). During this period, approximately 100.1 – 200.0 millimeter of rain were registered per month (Cptec-Inpe 2018) (see annex for more details). This shows that water-soluble compounds of litter can diminish rapidly due to leaching caused by rainfall and soil water flow as mentioned by Anderson (1973). The small increase of the total flavonoid content observed from T2 to T3 treatment might be due to the breakdown of leaf litter structure and release of structural compounds (such as lignin, carboxylic acids and amino acids) that occur in the late stages of decomposition due to fungal activity, but this can only be confirmed with a greater study period. However, given the fact that the referred increase from T2 to T3 was very small, it might also have been due to spectrophotometer limitation (equipment error).

Changes in chemical characteristics of leaf litter (phenolic content) might also occur due to the presence of microorganisms. Chomel et al. (2014) has shown that the activity of microorganisms, such as bacteria, decreases water soluble phenolic derivatives, because these organisms use simple molecules (*e.g.* simple sugars) as resource in the early stages of decomposition. Although it was not been evaluated in the present study, oxidation of phenolic derivatives might have also occurred due to the activity of microorganisms.

In the experiment which evaluated *P. scaber* feeding behavior on different decomposition stages of *C. illinoinensis* leaves, a tendency to higher consumption rate of three month old leaves and green leaves in comparison with other treatments was observed. However, the difference was not statistically significant. For this reason, the second hypothesis, that low phenolic and flavonoid content in the late stages of litter decomposition would stimulate the feeding behavior of *P. scaber*, was not statistically confirmed. Studying how phenolic content and leaf litter decomposition affect feeding rates of *Balloniscus sellowii* (Brandt, 1833), Wood et al. (2012) observed that isopods had high consumption rate when fed two month old leaves and low consumption rate when fed green leaves. However, this comparison must be done with caution due to differences between the two isopod species (*B. sellowii* is a neotropical species). Cameron and LaPoint (1978) showed that decrease in tannin content increased leaf litter consumption of *Armadillidium vulgare* (Latreille, 1804), suggesting that leaves with high tannin content (*e.g.* green leaves and/or recently fallen leaves) limit isopod behavior. Besides, authors showed that leaves with less tannin content (leached leaves) were more palatable and preferred by isopods. Over the years, many other studies have shown that food with a high phenolic content can deter detritivores from feeding on it (Poinsot-Balaguer et al. 1993; Zimmer 1999; Pennings et al. 2000) and that this limitation influences the decomposition process (Cameron and LaPoint, 1978; Savoie and Gourbie're 1989). Consequently, chemical changes occurred in food during decomposition increase its palatability and woodlice preference (Cameron and LaPoint 1978; Rushton and Hassall 1983; Pennings et al. 2000). In the present study, there was a tendency to higher consumption of three month old leaves by *P. scaber*, but this result may not be confirmed due to the number of isopods used in the experiment.

The observed high consumption of green leaves in the present study can be related to a forced experimental situation to provide a comparison at an initial stage (T0), as showed by Wood et al. (2012). Considering that *C. illinoinensis* is a deciduous species and *P. scaber* feeds on senescent litter from the ground, the consumption of green leaves might be occasional (green leaves fall due to the winds). Since green leaves were used as “control” (initial stage) to establish comparisons among other decomposition stages, this result was not expected. Isopods normally present higher consumption when leaves are more decomposed due to high palatability and low phenolic content as mentioned above. Furthermore, when isopods have a choice, they avoid consuming green leaves due to the high tannin content, as showed by Cameron and LaPoint (1978). Therefore, since *P. scaber* was used in a no choice experiment, the consumption of green leaves might have been due to hunger or to the fact that it had no choice.

On the other hand, the high quantity of phenolic derivatives found in green leaves did not inhibit *P. scaber* behavior in the present study. Similar results were found by Zimmer et al. (2005). They showed that *Porcellio dispar* (Verhoeff, 1901) consumed equally well *Eucalyptus globulus* (Labill.) and *Quercus faginea* (Lam.), two tree species that differed quantitatively in phenolic content (24 ± 4 mg tannic acid/g dry weight and 18 ± 8 mg tannic acid/g dry weight, respectively). That is, *P. dispar* did not have food-specific consumption rate. The authors also showed that *P. dispar* can digest both litter types equally well, while the same was not true to other isopod species evaluated (*Eluma caelatum*, Miers 1877 and *Proasellus coxalis*, Dollfus 1892). Although Zimmer et al. (2005) found a smaller amount of phenolic derivatives (calculi as tannic acid) than was found in the present study, *E. globulus* and *Q. faginea* are tree species known to limit leaf consumption by isopods through the presence of secondary metabolites. Studies have shown that phenolic compounds were found in green leaves as complex molecules (*e.g.* gallic acid glycosides, flavonoid polymers), and high concentrations of these compounds negatively affect isopods' feeding behavior and gut organisms (Cameron and LaPoint 1978; Harborne 1993; Zimmer 1999). However, Zimmer (1999) showed that *P. scaber* has the ability to break down (hydrolytically) these complex molecules (gallotannins), and this serves as a detoxification mechanism when they ingest food with high tannin content. Considering that the main food source of terrestrial isopods is decomposing plant litter (Van Wensem et al. 1993), and that cellulose represents a large part of carbon source for woodlice (Kozlovskaja and Striganova 1977), the ability to degrade complex molecules contributes to food utilization (Zimmer 1999; Zimmer et al. 2002). This ability to break down complex molecules might explain why *P. scaber* did not avoid green leaves consumption in the present study. Besides, they might have used broken down simple molecules (*e.g.* glucose) as resource.

Another factor that can contribute to the high consumption of green leaves by isopods is the role of phenolic derivatives in the choice of a particular food source. Although some studies have shown negative effects of phenolic derivatives on the behavior of insects (Lepidoptera)

(Hoffmann-Campo et al. 2001; Hoffmann-Campo et al. 2006; Salvador et al. 2010), the presence of phenolic derivatives can also act as a phagostimulant. Wood et al. (2012) suggested that flavonoids can possibly act as an antioxidant in isopods and, therefore, these organisms need to ingest a minimum quantity of them. However, more studies with isopods are necessary.

In the present study, *P. scaber* had a high consumption rate when feeding on three month old leaves, followed by green leaves. The egestion rate followed the same pattern as the consumption rate, with the highest egestion rates found in treatments with three month old leaves, followed by green leaves. Additionally, in the present study, the consumption rate and the egestion rate were significantly correlated. Wood et al. (2012) also found a correlation between egestion and consumption rates of *B. sellowii* when they fed on *Schinus terebinthifolius* Raddi. This correlation occurs (in part) due to the high quality of the food. Litter is more palatable and has less phenolic content. This is, the more isopods feed, the more they egest due to the quick passage of litter through the gut. The same might have occurred in the present study. This study also showed that assimilation rates were high for green leaves but low for three month old leaves. Similar results were showed by Sousa et al. (1998). They found high consumption and assimilation rates by *Porcellio dilatatus* (Brandt 1833) when it consumed pine. The authors attributed the results to the slow passage of litter through the gut or due to the adaptation of gut microbiota to litter. This can occur when isopods have that food in their natural habitat and can explain why the same results were found in present study for green leaves. The observed low assimilation rate for three month old leaves might be caused by the low nutritional value of leaf litter in late decomposition stages. With litter degradation, compounds of low molecular weight (*e.g.* simple sugar) are rapidly leached and/or released from leaves, while only structural compounds remain. In this case, *P. scaber* needed to increase consumption in order to obtain a minimum amount of nutrients. For Zimmer (2002), isopods need to feed on a great variety of litter to obtain their nutritional requirements, and this variety may not be found in a single food or, in this case, in a no choice experiment. Consequently, when isopods have low food resources, they can increase their consumption rates in order to satisfy their nutritional needs (Hassall and Rushton 1982). Although nutrient analysis is not the aim of this study, this factor might contribute to explain some results found here.

High mortality was found in treatments with green leaves and three month old leaves. In treatments with green leaves, the high content of phenolic derivatives might explain this result. Zimmer and Topp (2000) found similar results. The authors showed that consumption of litter with a high phenolic content increased mortality of *P. scaber* and *O. asellus*. Cameron and LaPoint. (1978) showed that the decrease in tannin content decreased mortality of *A. vulgare*, while in the present study, the highest mortality was found in treatment with three month old leaves. Considering that in this treatment the amount of phenolic derivatives is low, the high mortality might be due to other factors (age and stressful conditions). Rushton and Hassall (1983) attributed the mortality of isopods in their experiment to ‘other factors’, and not to phenolic content. Hassall and Rushton (1982) showed that mortality could still occur even under favorable conditions.

Although the experiment which evaluated the isolated effect of rutin and quercetin (flavonoids) in the feeding behavior of *P. scaber* (with agarose gel) showed no statistical difference in terms of consumption and assimilation rates, a slight tendency of increase according to increased rutin concentration offered to isopods was observed. This result might show a phagostimulant property of rutin, since this flavonoid might act increasing food consumption by *P. scaber*. The same was not observed in the experiment with quercetin. In this case, there was a slight tendency of decreased consumption when increased concentration of the compound was offered. This might show a phagoinibitory property of quercetin. Napal and Palacios (2015) showed that the consumption rate of *Spodopthera frugiperda* J. E. (Smith) (Lepidoptera, Noctuidae) did not differ between quercetin foods and control foods. The difference found in the present study between these flavonoids might be due to the chemical structure of rutin. Harborne (1993) showed that rutin and quercetin differ in chemical structures. Rutin (quercetin-3-O-rutinoside) is a glycoside of quercetin, so these substances have a great difference on their polarity and water solubility. This way, the glycoside in the chemical structure of rutin might make this flavonoid more palatable than quercetin, facilitating it consumption. Gazzoni et al. (1997) found that the consumption behavior of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera, Noctuidae) decreased with high concentrations of rutin and quercetin.

The present study found that mortality increased with increased concentrations of the flavonoids rutin and quercetin, being higher for concentration 2.0 mg/ml. Gazzoni et al. (1997) showed that mortality increased with increased concentrations of these compounds. A different result was found by Napal and Palacios (2015). They showed a small increase in mortality when *S. frugiperda* consumed quercetin. These differences might be due to the chemical structure, as showed by Harborne (1993), and due to exposure time, utilized concentration, utilized animal and the number of experimental units in the study. Since the results found with agarose gel were not statistically different (except for egestion rate of quercetin), the third hypothesis, that a gradual increase in the concentration of flavonoids (rutin and quercetin) in artificial foods would reduce the feeding rates of *P. scaber*, was not confirmed. We suggest that future studies should increase the number of experimental units used in this study.

As a conclusion, this study showed that the feeding behavior of *P. scaber* was not inhibited by the presence of total phenolic content and total flavonoid content in *C. illinoinensis* leaves. The experiment with rutin and quercetin did not found an optimal concentration that stimulates *P. scaber* feeding. Rutin and quercetin have the potential to modulate feeding behavior of *P. scaber*. However, more studies are necessary. It is important to point out that until now, this is the only study that evaluated monthly the total phenolic content and total flavonoid content in *C. illinoinensis* leaves and its effect in the feeding behavior of *P. scaber*.

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3 CONSIDERAÇÕES FINAIS

Os resultados desta dissertação mostram que o comportamento alimentar de *P. scaber* não foi inibido pela presença de alto teor de fenólicos totais e flavonoides totais presentes nas folhas de *C. illinoinensis*. É importante que estudos futuros avaliem o efeito da alta concentração de derivados fenólicos e da variação destas substâncias ao longo da decomposição foliar de *C. illinoinensis* em parâmetros populacionais de detritívoros, aspectos não avaliados neste estudo, como o efeito no tamanho das fêmeas e reprodução de *P. scaber*, uma vez que o consumo pode ocultar efeitos futuros na espécie.

Apensar das limitações de experimentos sem escolha alimentar, o presente trabalho pode evidenciar o papel detritívoro de *P. scaber*. Considerando que o isópodo não foi inibido pelos fenólicos e flavonoides, a espécie poderia estar contribuindo para uma rápida degradação e retomo dos nutrientes presentes nas folhas para o solo, favorecendo a ocupação de ambientes por outros animais ou plantas, entretanto, são necessários estudos mais aprofundados quando análise de nutrientes.

Sugerimos também estudos de múltipla escolha, oferecendo simultaneamente folhas de *C. illinoinensis* em diferentes estágios de decomposição e também experimentos com outras espécies vegetais, com altos e baixos teores de derivados fenólicos. Além de estudos sobre a metabolização do alimento e o efeito no solo e no desenvolvimento de plantas.

No experimento com gel de agarose o efeito dos flavonoides rutina e quercetina ficou mais evidente devido à possibilidade de verificar o efeito isoladamente. Quercetina teve efeito negativo mais evidente que rutina. O aumento na concentração de rutina parece estimular o consumo dos isópodos. Os dois flavonoides podem modular o comportamento de *P. scaber*.

Não foi possível identificar uma concentração ótima que estimulasse o comportamento alimentar de *P. scaber*. É importante que estudos futuros aumentem o tempo de exposição, o número amostral e as concentrações utilizadas no experimento.

É importante salientar que até o momento, este é o único trabalho que avaliou mensalmente o teor de fenólicos totais e flavonoides totais em folhas de *C. illinoinensis* e o efeito sobre o isópodo cosmopolita *P. scaber*. Sugerimos que estudos futuros avaliem o teor de derivados fenólicos em intervalos mais curtos e por mais tempo.

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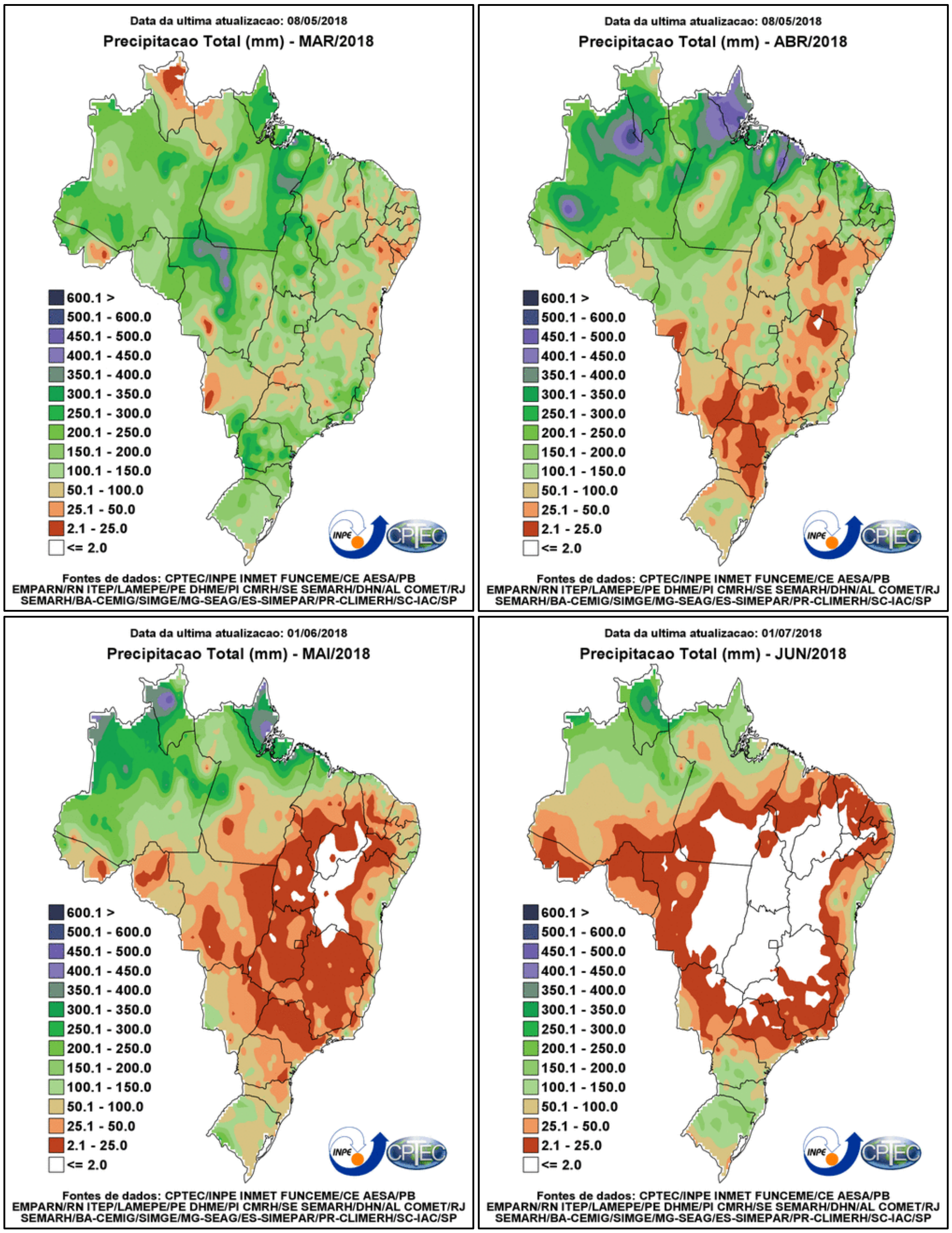
ANEXO – PRECIPITAÇÃO TOTAL PARA PORTO ALEGRE – RS, REFERENTE AOS MESES EM QUE OS LITTER BAGS FICARAM SOB CONDIÇÕES AMBIENTAIS



Fonte: Cptec-Inpe. **Previsão climática**. Disponível em: <https://clima1.cptec.inpe.br/monitoramentobrasil/pt>. Acesso 20 maio 2019.