UNIVERSIDADE FEDERAL DE LAVRAS

JESSICA JOSEFA SANCHES

EFFECT OF TEMPERATURE AND AGRICULTURAL MACHINERY ON ANT FORAGING INACTIVITY AND ROLE OF BODY SIZE ON METABOLIC RATE

LAVRAS - MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutora.

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EFEITO DA TEMPERATURA E MAQUINAS AGRICOLAS NA INATIVIDADE PARA O FORRAGEAMENTO E O PAPEL DO TAMANHO CORPORAL NA TAXA METABOLICA

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutora.

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RESUMO

As formigas são insetos extremamente complexos, e que apresentam alta plasticidade comportamental que permitiu com que se adaptassem à diversos ambientes. As formigas forrageiam num ritmo circadiano, mas esses padrões podem ser influenciados por inúmeros fatores, podendo inclusive causar a inativação de forrageamento por vários dias. Os fatores que causam esse comportamento ainda permanecem desconhecido. Entretanto, algumas hipóteses devem ser testadas uma vez que influenciam fortemente o comportamento de formigas. A primeira hipótese é que condições climáticas inadequadas podem causar a inatividade no forrageamento de formigas, como por exemplo a temperatura. De fato, a temperatura é um dos fatores que mais influenciam os padrões de forrageamento em formigas, por isso é necessário entender se esse fator é suficiente para causar a inatividade e como influencia no gasto de energia de operárias. Outra questão é que, uma vez que esses insetos possuem uma alta plasticidade comportamental, é compreensível acreditar que as formigas apresentem meios de lidar com a escassez de folhas após esse período de inatividade. Outro fator importante é o uso de maquinário agrícola, pois o dano causado pelo rodado do trator pode causar inatividade em detrimento da coleta de folhas, mas isso ainda precisa ser testado. Conhecer os a influencia desses fatores sobre a inatividade de forrageamento é essencial para o conhecimento do comportamento dessas formigas e seu uso no manejo dessas importantes pragas.

Palavras-chave: Formigas cortadeiras. Forrageamento. Comportamento de insetos.

ABSTRACT

Ants are insects extremely complex insects that have high behavioral plasticity in which allowed them to adapt to several environments. Ants forage at a circadian rhythm, but these patterns can be influenced by numerous factors, and may even cause foraging to be inactivated for several days. The factors that cause this behavior remain unknown. However, some hypotheses should be tested since they strongly influence the behavior of ants. The first hypothesis is that inappropriate weather conditions can cause inactivity in the foraging of ants, such as temperature. In fact, temperature is one of the factors that most influence foraging patterns in ants, so it is necessary to understand whether this factor is sufficient to cause inactivity and how it influences the energy expenditure of workers. Another issue is that, since these insects have a high behavioral plasticity, it is understandable to believe that ants present means of dealing with the scarcity of leaves after this period of inactivity. Another important factor is the use of agricultural machinery, as the damage caused by the tractor wheel can cause inactivity at the expense of leaf collection, but this still needs to be tested. Knowing the influence of these factors on foraging inactivity is essential for the knowledge of the behavior of these ants and their use in the management of these important pests.

Keywords: Leaf-cutting ants. Foraging. Insects behaviour.

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PRIMEIRA PARTE

1 INRODUÇÃO GERAL

As formigas estão entre os organismos mais abundantes na superfície da Terra estimativas consideram aproximadamente 20 quatrilhões de indivíduos distribuídos em diferentes biomas e ambientes (SCHULTHEISS et al., 2022). Ao longo da evolução, as formigas desenvolveram formas de adaptação à diferentes ambientes (MUELLER; REHNER; SCHULTZ, 1998), um dos quais está relacionado aos padrões alimentares. Por exemplo, algumas formigas podem se alimentar de sementes e plantas, e outras, embora também carreguem partes de plantas para o ninho, cultivam um fungo simbiótico que utilizam como principal fonte de nutrientes para suas larvas (QUINLAN; CHERRETT, 1979; SHIK et al., 2018). Independentemente da destinação que as formigas dão às partes das plantas, esses insetos são de extrema importância em um contexto econômico, uma vez que causam diversos danos às culturas, como por exemplo: cereais (BARAIBAR et al., 2011) e florestas plantadas (ZANETTI et al., 2003; SOUZA; ZANETTI; CALEGARIO, 2011).

Formigas, como as cortadeiras e coletoras, selecionam e transportam material vegetal para o ninho em um processo chamado forrageamento (MADEIRA-ROCHA; HUBBELL, 1987). Embora o forrageamento seja importante para a manutenção de seu suprimento de alimentos, alguns comportamentos intrigantes são mostrados em colônias, como a inatividade para forrageamento. A razão para a inatividade do forrageamento em algumas espécies é bem conhecida, por exemplo, formigas coletoras como *Messor* spp. podem apresentar paralisação no forrageamento durante o inverno na Europa (LOPATINA, 2018; JACQUIER et al., 2021). No Brasil, em geral, as condições climáticas não são extremas a ponto de causar uma inatividade sincronizada de todos os ninhos, porém ainda assim esses insetos apresentam inatividade no forrageamento em algumas colônias cuja razão ainda não está clara.

Uma das hipóteses para explicar a inatividade no forrageamento está relacionada à temperatura. As formigas são fortemente influenciadas pela temperatura (FOWLER; ROBINSON, 1979; VOGT et al., 2003; WELCH; BAUDIER; HARRISON, 2020), uma vez que são animais ectotérmicos e sofrem facilmente com a desidratação (MEAD-BRIGGS, 1956; CHOWN; SØRENSEN; TERBLANCHE, 2011; PERL; NIVEN, 2018). É razoável pensar que as formigas poderiam manter o forrageamento inativado em más condições de temperatura, principalmente em pequenos ninhos, porque o tamanho das formigas é geralmente pequeno (LEWIS; POLLARD; DIBLEY, 1974; FOWLER; ROBINSON, 1979). Porém, formigas forrageadoras maiores devem, teoricamente, permitir que uma colônia amplie sua atividade de forrageamento em temperaturas mais amplas. De fato, devido ao dimensionamento alométrico entre a superfície corporal e a massa corporal, os forrageadores maiores devem ter uma menor taxa de aquecimento e uma menor taxa de perda de água e, portanto, devem ser mais tolerantes a altas temperaturas do que os pequenos (EDNEY, 1977; AJAYI et al., 2020). Se as operárias maiores são mais tolerantes a baixas ou altas temperaturas, pode-se supor que a atividade de forrageamento de grandes colônias, nas quais a variação no tamanho das operárias é muito maior, deve ser menos dependente da temperatura ambiente do que a de pequenas colônias. As pequenas colônias seriam, portanto, mais vulneráveis à falta de alimento do que as grandes colônias em períodos prolongados de baixas ou altas temperaturas, explicando a elevada taxa de mortalidade observada nas colônias na sua fase inicial de desenvolvimento.

A tolerância térmica pode ser avaliada, por exemplo, medindo a Taxa metabólica (TM) das formigas (ROEDER; ROEDER; BUJAN, 2021), que corresponde à quantidade de energia que um indivíduo precisa para manter seu corpo funcionando (SHIK et al., 2019). A taxa metabólica em formigas polimórficas já foi medida para diversas espécies, tais como: *Anoplolepis steinergroeveri* (CHOWN et al., 2007), *Atta colombica* (CHOWN et al., 2007), *Camponotus* spp., (CHOWN et al., 2007; ÁGUAS, 2014), *Eciton hamatum* (CHOWN et al., 2007), *Formica rufa* (CHOWN et al., 2007), *Messor* spp. (CHOWN et al., 2007) e *Aphaenogaster iberica* (SHIK et al., 2019). Embora o valor da TM forneça informações significativas, é mais interessante expressar a TM por unidade de massa corporal (TM específica de massa), pois permite comparar a TM de animais de tamanhos muito diferentes em diferentes condições. O gasto de energia necessário para manter o corpo em diferentes temperaturas pode estar ligado à distribuição dos tamanhos das operárias e às diferentes tarefas atribuídas dentro das colônias de formigas. De acordo com a relação inversa entre a taxa específica de massa e o tamanho do corpo, é possível inferir que as operárias maiores precisam de menos energia por unidade de peso corporal em comparação com as menores e, portanto, podem dominar a força de trabalho de forrageamento. Além disso, grandes trabalhadores com uma menor proporção de área de superfície corporal para massa corporal podem suportar temperaturas mais altas, tornando-os mais adaptáveis. Portanto, as colônias de formigas que exibem uma gama mais ampla de dimensionadores de operárias provavelmente terão uma vantagem competitiva, pois podem forragear em faixas mais ampla de temperaturas. Essa abordagem deve ser mais explorada na literatura (SHIK et al., 2019).

Outra hipótese para explicar a inatividade de forrageamento das colônias poderia ser a necessidade das colônias por alimento. Colônias de formigas cortadeiras não forrageiam todos os dias, mesmo em condições de temperatura favoráveis e sem danos estruturais nos ninhos. Então, a falta de um padrão generalizado de forrageamento nessas condições pode ser devido à assincronia de requisitos nutricionais entre as colônias (FARJI-BRENER, 1993). Os estudos de forrageamento em formigas cortadeiras associam esta atividade aos fatores ambientais, entretanto, nenhum deles mensurou a necessidade da colônia por alimento influenciando a atividade de forrageamento. Colônias inativas por um longo período (jejum prolongado) correriam o risco de forragear em condições adversas de temperatura para abastecer-se de folhas? Elas carregariam mais fragmentos para compensar o período sem folhas?

Outra hipótese relacionada à inatividade do forrageamento de formigas é referente à perturbações mecânicas na estrutura do ninho, como aquelas causadas por grandes predadores (ex. tatus), pisoteio de grandes animais (ex. gado) e uso de máquinas agrícolas pesadas (ex. tratores). O uso de máquinas agrícolas em áreas cultivadas, como as plantações de eucalipto, influencia a atividade de forrageamento das colônias por meio de mudanças na estrutura física do solo (LEAL et al., 2014). As formigas cortadeiras constroem uma cadeia de túneis abaixo do solo (MOREIRA et al., 2004), que são essenciais para manter o microclima interno dos ninhos adequados para o cultivo do fungo simbionte (BOLLAZZI; ROCES, 2007; BOLLAZZI; KRONENBITTER; ROCES, 2008; FORTI et al., 2011). O uso de máquinas agrícolas provavelmente danifica a estrutura dos túneis e compromete a integridade dos jardins de fungos. Assim, depois que os ninhos são severamente danificados, talvez mais formigas sejam realocadas para tarefas de escavação em detrimento do forrageamento, levando a uma diminuição acentuada ou interrupção completa da atividade de forrageamento por alguns dias. A influência do maquinário na inatividade de forrageamento das formigas ainda é desconhecida, bem como seu comportamento após esse período.

Neste contexto, as pesquisas sobre como os fatores climáticos e antrópicos influenciam a inatividade de forrageamento das colônias permitem aperfeiçoar as estimativas de herbivoria e as estratégias de controle de formigas cortadeiras, aumentando a eficiência do controle, minimizando o desperdício e contaminação ambiental.

2 RFERENCIAL TEÓRICO

2.1 Forrageamento em formigas

As formigas coletoras e cortadeiras são conhecidos por seu comportamento distinto de forrageamento. As formigas coletoras transportam sementes, grãos e outras fontes de alimento que podem ser armazenadas em seus ninhos para uso posterior (TRANIELLO, 1989; BEVERLY et al., 2009). Enquanto isso, as formigas cortadeiras cortam pedaços de folhas de árvores e plantas, transportando-as de volta ao seu ninho para cultivar fungos, que constituem sua principal fonte de alimento (WILSON, 1980). Ambas as espécies são altamente organizadas trabalhando coletivamente para forragear, transportar e armazenar alimentos. Esse comportamento altamente especializado é uma das muitas maneiras pelas quais as formigas são capazes de sobreviver e prosperar em uma ampla variedade de ecossistemas em todo o mundo.

A especialização na coleta de vegetação vem fazendo com que as formigas colhedoras e cortadeiras causem danos nas lavouras. *Messor barbarus*, uma importante formiga coletora, por exemplo, está relacionada com a redução em 9,2% das perdas em lavouras de cereais (BARAIBAR et al., 2011). As formigas cortadeiras do gênero *Atta* podem transportar uma enorme quantidade de folhas, como *Atta capiguara* com 150 kg de biomassa seca por ano (CALDATO et al., 2016); *Atta volenweideri* com 90 a 250 kg/colônia/ano em áreas nativas do Paraguai (FOWLER et al., 1986); e cerca de 1216,96 kg de peso seco de grama/ano em pastagens cultivadas na Argentina (GUILADA; FOLGARAIT, 2015a); *Atta laevigata* pode coletar cerca de 172,9 kg/colônia/ano de material vegetal no Cerrado (COSTA et al., 2008); *Atta bisphaerica* provoca uma queda na produtividade de 3,2 t/ha na cana-de-açúcar, correspondendo a 5,3% da produtividade (PRECETTI et al. 1988).

A seleção e exploração de recursos por formigas é um processo sofisticado, exigindo um comportamento refinado de identificação e escolha para garantir o crescimento saudável da colônia (MUNDIM; COSTA; VASCONCELOS, 2009; RIACHO; MARINHO, 2011). As plantas são selecionadas com base nas propriedades físicas e químicas das folhas, de maneira que as formigas cortadeiras, por exemplo, preferem folhas com baixo nível de umidade, uma vez que essas folhas possuem menos mecanismos de defesa mecânica e química, mas com alto valor nutricional (ROCES; HÖLLDOBLER, 1994; VALÊNCIA; CHERRETT, 1996; MEYER; ROCES; WIRTH, 2006). Os voláteis emitidos pelas plantas e a ingestão de seiva são essenciais para o reconhecimento de componentes de baixa volatilidade, como antidigestivos ou compostos secundários tóxicos do grupo tanino, como observado para *A. sexdens* e *A. laevigata*.

(SANTANA; COUTO, 1990; HERZ; HÖLLDOBLER; ROCES, 2008; SAVERSCHEK; ROCES, 2011). O cultivo do fungo é essencial para a sobrevivência da colônia e por isso as formigas desenvolveram estratégias para evitar danos ao fungo, como por exemplo a rejeição de fragmentos com alguma característica fungicida ou formicida após a incorporação dos fragmentos no jardim do fungo, como observado para *Acromyrmex lundii* (Guérin-Meneville) (NORTE; HOWSE; JACKSON, 2000; HERZ; HÖLLDOBLER; ROCES, 2008).

O corte e o transporte por formigas cortadeiras são realizados após a seleção do material vegetal, todas as partes da planta são aproveitadas por elas, tais como: folhas, frutos, flores e caule (RIBEIRO; MARINHO, 2011). Esses insetos apresentam diversas estratégias para tornar o processo de coleta e transporte mais rápido e eficiente, devido à necessidade de percorrer longas distâncias para coletar recursos (FOWLER; ROBINSON, 1979). O particionamento de tarefas é uma dessas estratégias, na qual formigas menores cortam e derrubam folhas de árvores, e as maiores carregam fragmentos, como foi observado em *Atta colombica* (TOLEDO et al., 2016), *A. sexdens* (FOWLER; ROBINSON, 1979; TOLEDO et al., 2016), *Atta vollenweideri* (RÖSCHARD; ROCES, 2003), mas não em *A. laevigata.* (LIMA et al., 2006).

Transportar os fragmentos coletados é uma etapa crucial para as formigas cortadeiras, pois elas adaptam o tamanho da carga para maximizar a eficiência do seu forrageamento (BURD, 2000; GARRETT et al., 2016). Há uma grande flexibilidade na escolha do tamanho dos fragmentos carregados e no recrutamento de formigas, o que se adequa às diversas condições encontradas nos percursos de busca por substrato (FOWLER; ROBINSON, 1979; FOURCASSIE; DUSSUTOUR; DENEUBOURG, 2010).

Os fatores que influenciam o forrageamento das formigas são numerosos e complexos. A disponibilidade de alimentos é de grande importância, mas não é o único fator que determina sua estratégia de busca. A temperatura (FOWLER; ROBINSON, 1979; GIESEL; BOFF; BOFF, 2013; RAMIREZ-OLIER et al., 2022), a umidade (LIGHTON; BARTHOLOMEW, 1988a) e a presença de predadores e competidores (GUILLADE; FOLGARAIT, 2015b) também têm um papel importante na determinação de como e onde as formigas buscam alimentos. A habilidade das formigas em se adaptar e mudar suas estratégias de forrageamento depende desses fatores, permitindo que elas encontrem recursos quando as condições são favoráveis (FOWLER; ROBINSON, 1979; VIANA-BAILEZ; ENDRINGER, 2016). Além disso, a coordenação e cooperação dentro da colônia é essencial para aumentar a eficiência da busca de recursos, resultando em uma alta taxa de sucesso em uma ampla variedade de ambientes (FOWLER; ROBINSON, 1979). Em resumo, a capacidade das formigas em forragear é influenciada por uma série de fatores, mas sua habilidade de adaptação e cooperação lhes permite prosperar em várias situações.

Diversos fatores podem influenciar no forrageamento de formigas, principalmente fatores ambientais como: temperatura (FOWLER; ROBINSON, 1979; VOGT et al., 2003; LIMA et al., 2006), umidade (LIGHTON; BARTHOLOMEW, 1988b), presença de inimigos naturais (BRAGANÇA; TONHASCA; DELIA LUCIA, 1998; GUILLADE; FOLGARAIT, 2015b), vento (ALMA; FARJI-BRENER; ELIZALDE, 2016a, 2016b) e chuva (FARJI-BRENER et al., 2018). Dentre estes fatores, destaca-se a temperatura pois afeta direta e indiretamente sua atividade.

A temperatura é limitante para o forrageamento de formigas, podendo aumentar ou ser completamente cessado dependendo das condições favoráveis ou desfavoráveis de temperatura, como ocorre para *Atta capiguara* que apresenta maiores taxas de forrageamento em temperatura próxima à 25ºC e para *A. sexdens* que cessa completamente o seu forrageamento em temperaturas abaixo de 10 e acima de 30°C (FOWLER; ROBINSON, 1979; CALDATO et al., 2016). Além disso, o forrageamento não ocorre de maneira regular, sendo mortificado de acordo com as estações e até mesmo ao longo do dia. O forrageamento de *A. sexdens* e *A. cephalotes* ocorre principalmente durante o dia no inverno e durante a noite no verão (FOWLER; ROBINSON, 1979). Já a atividade de forrageamento de *A. colombica* (WIRTH et al., 2003) e *Acromyrmex subterraneus subterraneus* (MACIEL et al., 1995; NICKELE et al., 2016) ocorrem sobretudo durante o dia e noite, respectivamente. A temperatura é um fator decisivo para o forrageamento de alimento em diversas espécies, como em *A. crassispinus* e *A. subterraneus subterreneus* em que o forrageamento é mais intenso na primavera e verão (NICKELE et al., 2016). Resultados semelhantes foram verificados para *Atta sexdens priventris* que apresenta maior atividade de forrageamento quando a temperatura varia em torno de 26 ºC e ocorre em horários diferentes de acordo com a estação do ano (GIESEL; BOFF; BOFF, 2013). As formigas evitam forragear em temperaturas adversas em períodos específicos do dia ou do ano, pois essas condições são letais e impactam na sua fisiologia. (JAYATILAKA et al., 2011). A variação dos padrões de forrageamento depende da termotolerância das espécies de formigas, que está intimamente relacionada com a capacidade das operárias em explorar os ambientes, da distribuição geográfica desses insetos e da variação diário e sazonal do forrageamento (JAYATILAKA et al., 2011; DIAMOND et al., 2013; LOPES et al., 2016).

A medida da reação térmica de formigas individuais, ou seja, o padrão de variação de sua resposta ao aumento das temperaturas, pode ser avaliada pela taxa metabólica (TM), que corresponde à quantidade de energia que um indivíduo precisa para manter seu corpo funcionando (SHIK et al., 2019). A taxa metabólica em formigas varia de acordo com a temperatura (VOGT; APPEL, 1999; SHIK et al., 2019), tamanho das operárias (LIGHTON, 1994) e atividade(SHIK et al., 2019).

Medições de taxa metabólica em espécies de formigas que mostram variação do tamanho da operária já foram realizadas em diversas espécies, como: *Anoplolepis steinergroeveri* (CHOWN et al., 2007), *Atta colombica* (CHOWN et al., 2007), *Camponotus spp.* (CHOWN et al., 2007; WATERS, 2014), *Eciton hamatum* (CHOWN et al., 2007), *Formica rufa* (CHOWN et al., 2007), *Messor* spp. (CHOWN et al., 2007), *Formica rufa* (PERL; NIVEN, 2018) and *Aphaenogaster iberica* (SHIK et al., 2019). Em todos esses estudos, a taxa metabólica demonstrou aumentar alometricamente com o tamanho do corpo. No entanto, o expoente da equação que descreve a relação alométrica entre TM e tamanho corporal varia muito entre as espécies. Por exemplo, em *Messor pargandei* o valor da inclinação é 0,61, enquanto que em *M. capensis* é 1,28 (Chown et al. 2007) e em *Formica rufa* (Perl and Niven 2018) situa-se entre 0,46, e 0,59. Além disso, a taxa metabólica pode variar de acordo com a temperatura e localidade onde a espécie é encontrada (SHIK et al., 2019).

A umidade relativa do ar influencia a sensibilidade de formigas sobre condições desfavoráveis de temperatura, principalmente as altas, como observado para *Formica exsecta*. que apresenta maior mortalidade de operárias a 25°C que 20°C quando as condições de umidade estão próximas a 50% (STUCKI; FREITAK; SUNDSTRÖM, 2017). A umidade relativa do ar combinada com a temperatura determina valores de déficit de pressão de vapor de água (VPD), que basicamente é a diferença entre a pressão real do vapor de água no ar e a pressão de vapor de água de saturação a uma determinada temperatura, e isso pode indicar a capacidade de dessecação dos insetos (BUCK, 1981; CALDATO et al., 2016). Altos valores de VPD evitam a dessecação de formigas em altas temperaturas, em estações úmidas esses valores ficam sempre abaixo de 2 kPa, o que não ocorre nas estações secas, fazendo com que atividade de formiga *A. capiguara* reduzem abruptamente em estações com baixas umidades (CALDATO et al., 2016). A dessecação das formigas cortadeiras em condições de baixa umidade causa a morte das operárias e por isso essas formigas são adaptadas a forragear em condições ótimas, como a *A. capiguara* que forrageariam entre 50 a 70% de umidade (CALDATO et al., 2016), *Ac. subterraneus subterraneus* e *Ac. crassipinus* entre 60 a 80% (NICKELE et al., 2016).

Outro fator que pode influenciar o forrageamento por formigas é o dano mecânico a estrutura do ninho. As formigas redirecionam o gasto de energia para o reparo do ninho em detrimento do forrageamento (FARJI-BRENER; TADEY, 2012). Formigas gastam alta quantidade de energia para construir, reparar e manter a estrutura dos ninhos e consequentemente manter as condições microclimáticas ideais para o fungo (BOLLAZZI; ROCES, 2007, 2010). Danos na estrutura dos ninhos causam mudanças no comportamento de formigas cortadeiras (FARJI-BRENER; TADEY, 2012). Essa capacidade de responder as variações das condições externas ao ninho com mudança de comportamento é chamada de plasticidade comportamental (CHESSON, 1986). As formigascortadeiras reparam as colônias danificadas com materiais recusados, presentes principalmente no lixo do ninho. Foi observado que o reparo ocorre principalmente em estações quentes, pois o jardim de fungo fica mais vulnerável e suscetível a sofrer danos (FARJI-BRENER; TADEY, 2012). O aumento da mortalidade das colônias, o abandono do ninho e a modificação do tipo de plantas coletadas também foram comportamentos verificados após a danificação dos ninhos (FARJI-BRENER, 2000; FARJI-BRENER; TADEY, 2012).

2.2 Inatividade no forrageamento em formigas

Os insetos, bem como outros animais, podem apresentar inativação do forrageamento quando em condições desfavoráveis, seja de temperatura, excesso de chuvas ou até escassez de alimento (GIESEL; BOFF; BOFF, 2013). Algumas formigas, por exemplo, apresentam o comportamento de diapausa em que a colônia de formigas reduz sua atividade para conservar energia (KIPYATKOV; LOPATINA, 2009; LOPATINA, 2018). As formigas operárias permanecem dentro do ninho e cuidam da rainha e das novas gerações enquanto esperam que as temperaturas mais favoráveis cheguem (KIPYATKOV; LOPATINA, 2009).

A temperatura é um fator limitante para diversas espécies de formigas, permitindo o forrageamento apenas em faixas de temperatura específicas (DIAMOND, 2017; ESCH et al., 2017; ROEDER; ROEDER; BUJAN, 2021). *Atta sexdens*, por exemplo forrageiam em temperaturas entre 10 e 30°C (GIESEL; BOFF; BOFF, 2013), outras espécies que são encontradas principalmente em locais mais frios, não forrageiam em temperaturas muito baixas, como por exemplo abaixo de 0°C (LYNCH; BALINSKY; VAIL, 1980). *Prenolepis imparis* apresenta um período de estivação durante os meses quentes do verão, que pode variar de 3 a 8 meses em climas mais quentes e úmidos como a Flórida (TSCHINKEL, 1987). Durante esse tempo, a colônia quase não apresenta atividade de forrageamento e vive exclusivamente dos alimentos armazenados nas colônias (TSCHINKEL, 1987). Durante os meses de verão ou durante condições climáticas extremas, as formigas coletoras passam por um estado de diapausa conhecido como estivação/inatividade (LÓPEZ; SERRANO; ACOSTA, 1992; DOBLAS-MIRANDA; REYES-LÓPEZ, 2008). Da mesma maneira, as formigas cortadeiras, que pertencem aos gêneros *Atta* e *Acromyrmex*, são conhecidas por sua complexa estrutura social e impressionantes habilidades de forrageamento (VIANA-BAILEZ; ENDRINGER, 2016). Particularmente durante o inverno, quando a temperatura cai o forrageamento se torna inviável e os recursos se tornam escassos, as formigas que são adaptadas a estocar alimentos se sobressaem nesses ambientes por poder ficam tempos maiores sem coletar recursos (LYNCH; BALINSKY; VAIL, 1980; KIPYATKOV; LOPATINA, 2009).

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SEGUNDA PARTE

Normas do periódico Agricultural and Forest entomology (versão preliminar)

Artigo 1 - Factors influencing the cycles of foraging activity and inactivity in colonies of the leaf-cutting ant *Atta sexdens* (Hymenoptera: Formicidae) in Eucalyptus plantations

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ABSTRACT

The foraging activity of leaf-cutting ants is characterized by alternating between periods of inactivity and activity that spans several days, or weeks. Therefore, many factors could influence it, once, leaf-cutting ants are strongly affected by environmental factors, such as temperature, humidity, and rain. Likewise, the cycles of activity/inactivity in foraging could be modified by the colony size and use of agricultural machinery. The objective of this work was to evaluate the factors which influence the probability of foraging inactivity. Twenty transepts, with around 100 colonies of *Atta sexdens* each one, were selected and the nests were identified in eucalyptus plantations every two months per 1 year. Thus, we evaluated if foraging inactivity is influenced by size, temperature, humidity, rain, number of favorable days for foraging and use of machinery. The data were analyzed with glm modeling with distribution of binomial errors, using the software R. No abiotic and anthropic factor affected the proportion of inactive colonies, only the size of the colonies seems to be related to the inactivity cycle of leaf-cutter ants. The nest size influenced the probability of foraging inactivity, being higher in smaller nests, because they have smaller number of workers and consequently have lower capacity for competition and cannot maintain foraging. In addition, the workers produced by large colonies are bigger, more resistant to temperature and humidity, and more efficient in the division of labor, and in foraging than smaller ones. Leaf-cutting ants are one of the main herbivores in Brazil, often reaching pest status in several crops in the country. Knowledge about inactivity in foraging leaf-cutting ants is essential for the adoption of management techniques and also to understand their intraspecific relationships.

Keywords: leaf cutter ants, ants’ behavior, agricultural machinery

INTRODUCTION

Leaf-cutting ants of the genus *Atta*, *Acromyrmex* and *Amoimyrmex* count among one of the most fascinating insects in the world. They practice a sort of primitive agriculture: they cut and collect vegetation in the environment and bring it back to their nest to feed a symbiotic fungus that they use as their main source of food (Holldobler and Wilson 2010). These ants can form huge colonies of several hundred thousand individuals and the volume of underground nests of mature colonies can sometimes occupy more than one hundred cubic meters (Moreira et al. 2004). Because of their defoliation action, they can cause considerable damage to food and industrial crops (e.g. sugarcane, Eucalyptus) and they are thus considered the main herbivore in the Neotropical region and a major pest for cultivated plants (Della Lucia et al. 2014; Zanetti et al. 2014).

The foraging activity of leaf-cutting ants follows a circadian rhythm (Rockwood 1975; Caldato et al. 2016) which is influenced by several external factors, such as air temperature and humidity (Giesel et al. 2013; Tizón et al. 2014), the occurrence of rain (Farji-Brener et al. 2018) or wind (Alma et al. 2016), as well as the presence of natural enemies such as parasitoids (Guillade and Folgarait 2015). Leaf-cutter ants also show a high plasticity in their activity rhythm. Therefore, the same species can have different patterns of foraging activity depending of the type of environment in which it is found, e.g. forests, savannas, pastures (Kost et al. 2005; Urbas et al. 2007). However, over a time scale of several months, the foraging activity of leaf-cutting ants is characterized by an alternance of periods of activity and inactivity of several days, or even weeks, whose origin remains unknown. The fact that these periods are not necessarily synchronized between the colonies located in the same area is particularly intriguing. In cultivated areas such as pastures or planted forests where leaf-cutting ants are common, human disturbances could also play a role in determining the cycle of activity/inactivity. In planted forests for example tractors are used several times a year for the management of soil, the treatment of weed, and the distribution of chemical baits. However, the effects of frequent use of this disturbance on leaf-cutting ant foraging has so far not been documented.

In this paper we present a study that tests the hypothesis that both environmental and anthropic factors could be responsible for the alternance of foraging activity/inactivity in leaf-cutting ants. First, we hypothesize that on a seasonal time scale leaf-cutting ants are less likely to be inactive for several days after a long period of rainfall (Farji-Brener et al. 2018) or when the air temperature and humidity have been above or below certain values for a long period of time (Fowler and Robinson 1979; Giesel et al. 2013). Second, we hypothesize that in cultivated areas such as Eucalyptus plantations, anthropic factors could also influence the foraging activity of the colonies, for example through a change in the physical structure of the soil (Leal et al. 2014). Indeed, leaf-cutting ants build an extensive network of underground tunnels (Moreira et al. 2004) that are essential for maintaining the internal microclimate inside their nest that is suitable for the cultivation of their symbiotic fungus (Bollazzi and Roces 2007; Bollazzi et al. 2008). The use of agricultural machinery in cultivated areas is likely to damage the structure of these tunnels and therefore to compromise the integrity of the fungus garden. Hence, after the nest has been severely damaged, more workers may be relocated to excavation tasks (Farji-Brener and Tadey 2012) at the expense of foraging, leading to a sharp decrease or a complete stop of foraging activity during several days. Identifying the factors responsible for the cycles of foraging activity/inactivity could help to promote better control strategies that minimize waste and environmental contamination and to better predict the damages caused by leaf-cutting ant colonies in cultivated areas.

METHODOLOGY

Studied site

The leaf-cutting ant colonies we studied were located in six different plots of eucalyptus plantations in an area of Atlantic Forest situated in the Eastern part of the state of Minas Gerais, Brazil (Figure 1). All plots had not been chemically or mechanically treated for one year. Each plot was surveyed only once between July 2020 and July 2021.

Factors influencing foraging inactivity

In each plot of Eucalyptus plantation twenty 12 x 100m transects were delimited and the number of *A. sexdens* nests found inside these transects was counted. Each nest was georeferenced and its size was measured by the larger rectangular area that included all the mounds of loose excavated soil above the nests (Grandeza et al. 1999). In June, July, September and December 2020 and January and May 2021 the foraging activity of each nest was then evaluated by using the methodology proposed by (Ramos 2002), which consists in placing plastic baits coated with a powder made from citric pulp close to the nests and by subsequently observing whether these baits are rejected with excavated soil on the mound of loose soil above the nest. A colony was considered active if ants rejected the baits within 24 hours after their placement, otherwise it was considered inactive. The temperature, humidity and rainfall were obtained from the meteorological data of the closest weather station to the surveyed plots (Figure 1).

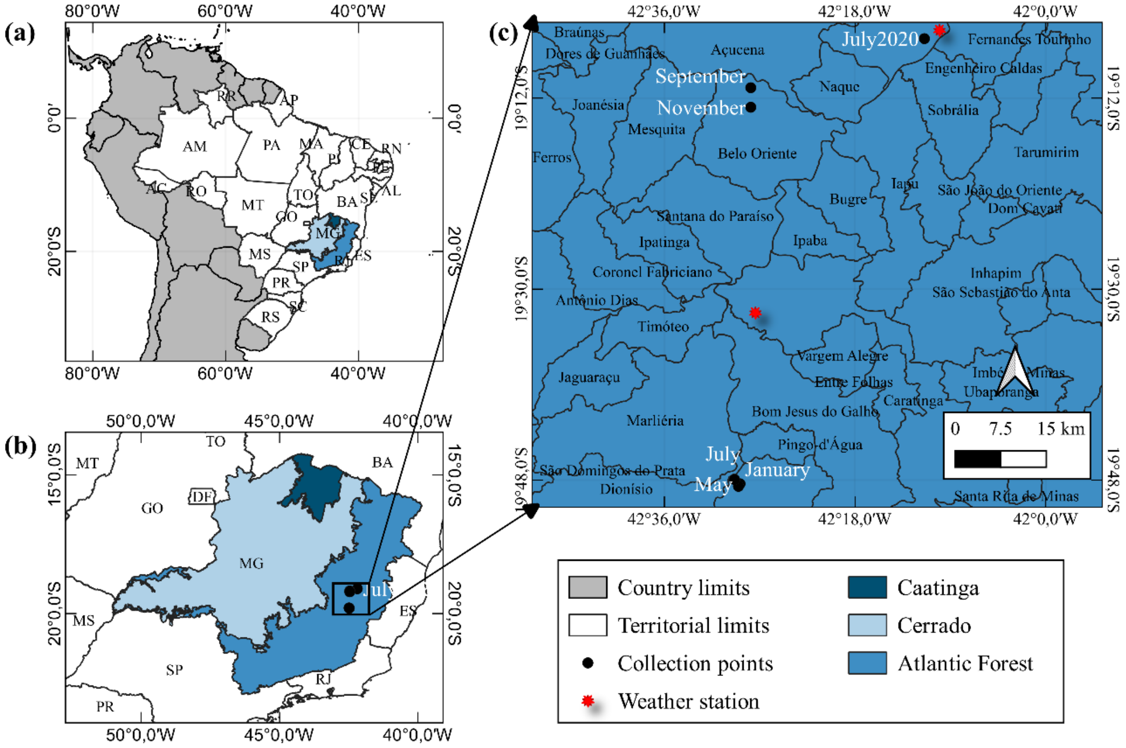

Figure 1. Maps showing the location of the plots of Eucalyptus forest where the studied Atta sexdens *colonies were located in Brazil (a), in the state of Minas Gerais (b) and in the area of Atlantic forest located in the eastern part of Minas Gerais (c).*

These latter were used to calculate the number of hours in which the meteorological conditions were favorable for the foraging activity of *A. sexdens* colonies on the day preceding our evaluation. We reasoned that foraging activity was more likely to be observed on the day of the evaluation if the meteorological conditions had not been favorable for foraging on the previous day. Based on a previous survey of the foraging activity of *A. sexdens* colonies (Abreu 2015), we considered that foraging activity was the more likely to have occur during an hour if the temperature was in the range of 18°C - 23°C and if no rainfall had occurred. Our previous activity survey shows that in these conditions the probability to find ants carrying vegetation back to their nest on foraging trails reaches at least 25%, half the maximum value observed.

A logistic regression was used to investigate the influence of the size of the nests, the number of favorable hours for foraging activity in the day preceding the survey of foraging activity and the impact of abiotic factors (mean temperature on the day of the survey, number of favorable hours for foraging) on the probability for the colonies of being inactive. Relative humidity was not considered in the analysis because it is highly correlated with temperature and generally not a relevant abiotic factor to explain foraging activity in leaf-cutting ants. We also tested the interaction between nest size and temperature because we reasoned that the probability of being inactive could vary differently with temperature in colonies of different sizes, with small colonies more likely to be inactive at high temperatures than large ones. In fact, worker size polymorphism is known to vary with colony age, with large colonies having on average larger workers and a higher size polymorphism than small colonies (Wilson 1980). Since there is a link between worker size and thermotolerance in leaf-cutting ants (Ribeiro et al. 2012; Bouchebti et al. 2014; Baudier and O'Donnell 2020), large colonies could thus be active for a larger temperature range than small colonies.

Influence of forest management practice on foraging activity

In May 2021 twenty additional transects were delimited in one of the plot of Eucalyptus plantation that was used for the evaluation of foraging activity in the experiment described above (Figure 1). The colonies of *A. sexdens* found in these transects were counted, georeferenced and their size was estimated by the area covered by the mounds of loose soil over the nest. The foraging activity of each nest was then assessed in the same way as described above. Then, after 24 hours, a tractor weighing 3500kg drove over the whole area of the transects, there was not any implement attached to the tractor, only the its weigh was considered to damage the colonies. The driver of the tractor was asked explicitly to pass over all leaf-cutting ant nests in the transects.

To test the effect of the passage of the tractor on the probability of a nest being inactive we also used a binomial GLMM. The chambers of small nests of *A. sexdens* are much closer to the soil surface than those of large nests (Camargo et al. 2016). Therefore, small nests are probably more likely to be affected by the passage of the tractor than large ones and the probability of being inactive as a function of size mays vary differently before and after the passage of the tractor. Consequently, we included the following independent variables in the model: tractor passage (with two levels: control – before the passage of the tractor, treatment – after the passage of the tractor), nest size and the interaction between these two variables. The nest identity was included as a random variable in the model. The day after the passage of the tractor we checked again with the method described above whether the nests were active or inactive.

All analyses were performed and graphics generated with R 3.4.1. The package ggplot2 (Wickam et al. 2022) was used to generate the graphics. The package lme4 was used to perform the GLMMs (Bates et al., 2015), the package DHARMa (Harting and Lohse 2022) to check the quality of logistic regression and the packages survival (Therneau and Lumley 2011) to perform the survival analyses.

RESULTS

Evaluation of foraging inactivity

There was no statistically significant influence of the mean daily air temperature on the day of foraging activity evaluation on the occurrence of foraging inactivity (Table 1). On the other hand, nest size had a statistically significant influence on the probability of observing foraging inactivity: small nests were more likely to be inactive than large ones (Figure 2).

Table 1. Influence of nest size, temperature and the number of hours favorable to foraging on the day preceding the evaluation of foraging activity on the occurrence of inactivity in A. sexdens *nests.*

	Df	Residual deviance[a]	*P*[b]
Nest size	1	9.8707	**0.001679**
Mean temperature	1	0.0028	0.957790
Mean temperature : Nest size	1	0.1159	0.733500
Number favorable hours : Nest size	1	0.5826	0.445285
Number favorable hours	1	6.2074	**0.012722**

[a] *GLM (family: binomial; link: logit; response: inactive [=1], active [=0]).*

[b] *p-values lower than the significance threshold of 0.05 are indicated in bold.*

Although the number of favorable hours for foraging on the day preceding the survey evaluation of foraging activity had a statistically significant effect on the occurrence of foraging inactivity (Table 1): the colonies were slightly more likely to be inactive if the number of favorable hours was reduced (Figure 3).

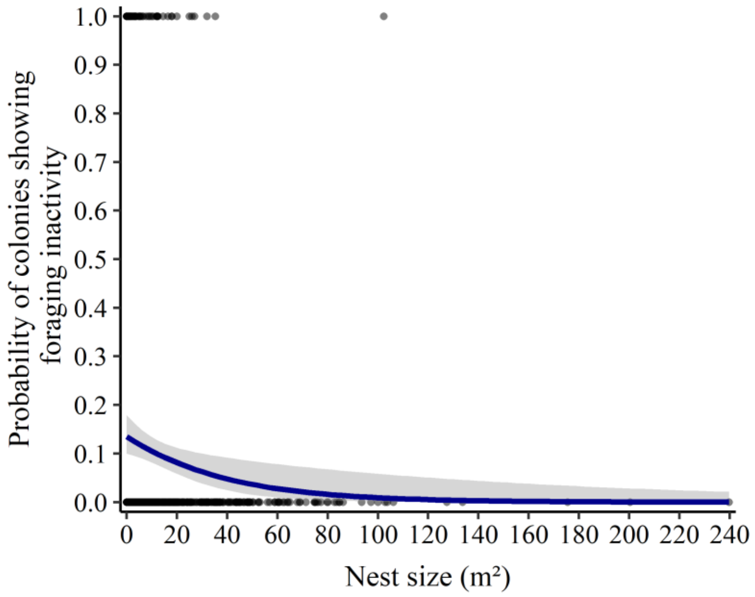

Figure 2. Probability of Atta sexdens *colonies showing foraging inactivity as a function of nest size. The grey area shows the confidence interval of the predictions. N= 579 nests.*

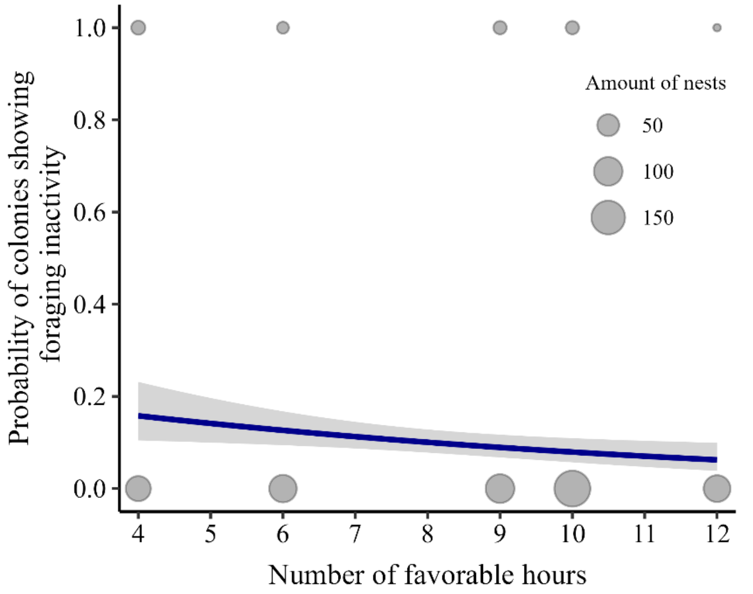

Figure 3. Probability of Atta sexdens *colonies showing foraging inactivity as a function of the number of hours favorable for foraging on the day preceding the evaluation of foraging activity. The grey area shows the confidence interval of the predictions. The size of the points is proportional to the number of nests with the corresponding value. N=579 nests.*

Influence of forest management practice on foraging activity

There was no significant interaction between the size of the colonies and the fact that the nests had been damaged. In other words, the damages caused by the tractor had no impact on the occurrence of foraging inactivity, regardless of colony size. Moreover, there was no relationship between the damages caused by the tractor or the size of the nests on the occurrence of inactivity of *A. sexdens* (Table 2).

Table 2. Influence of forest management practice on the occurrence of inactivity in Atta sexdens *nests*

	Df	Residual deviance[a]	*P*[b]
Nest size	1	1.3488	0.2455
Tractor passage	1	0.6562	0.4179
Nest size x Tractor passage	1	2.4446	0.1179

[a] *GLM (family: binomial; link: logit; response: inactive [=1], active [=0]).*

[b] *p-values lower than the significance threshold of 0.05 are indicated in bold.*

DISCUSSION

Leaf-cutting ants are some of the most interesting insects that exhibit pauses or inactivation in their foraging. Those pauses have been related to the weather conditions, as reported in species of ants in Europe, in which they hibernate due to the low temperatures in the winter and scarce of resources (Jacquier et al. 2021). In this work, we found that leaf cutting ants inactivity is not related to the weather, neither temperature nor number of favorable hours were significant to cause inactivation in ants foraging. In other hands, internal factors of the colonies, such as size of the nests must drive foraging inactivity. Also, the effects of the use of machinery on the field do not affect leaf-cutting ant foraging activity.

Temperature has been shown to be a limiting factor for foraging in leaf-cutting ants, with colony foraging activity being observed only within a given range of ambient temperatures (Giesel et al. 2013; Bouchebti et al. 2015; Baudier and O'Donnell 2020; Welch et al. 2020).

Changes in temperature can also modulate foraging activity by affecting the intensity of worker flow on foraging trails (Giesel et al. 2013), the speed and size of foraging workers (Burd 1996; Ramirez-Olier et al. 2022), the proportion of foraging workers carrying leaf fragments and thus the rate of vegetation biomass carried to the nest (Endringer et al. 2012). Nevertheless, we found no influence of ambient temperature on the foraging activity of *A. sexdens* colonies in the present study. *A. sexdens* is widely distributed in Brazil and it is characterized by a high thermotolerance. This should allow this species to forage within a wide range of temperatures, both in rural and urban areas (Angilletta et al. 2007; Roeder et al. 2021). The range of ambient temperatures to which the colonies were exposed during our survey may have been too restricted to have a significant impact on foraging activity.

The fact that the probability of being inactive was higher for small colonies compared to large ones could be linked to the size of the workers in the colony. In fact, in ant species like leaf-cutting ants showing a strong size polymorphism of the worker caste, the range of worker sizes varies with colony age and thus with colony size (Fowler and Robinson 1979). The average size of the workers in young colonies is smaller than in old colonies and there is also less variation in the size of foraging workers (Fowler and Robinson 1979). This may restrict the range of temperatures at which the colonies can forage. In fact, large workers, because of their lower surface to volume ratio are more tolerant to low or high temperatures than small workers (Bujan et al. 2016; Perl and Niven 2018; Packard 2020). Therefore, the foraging activity/inactivity of small colonies should be more dependent on ambient conditions than that of large colonies. Another factor that could affect the foraging activity of small nests is competition. In fact, the density of *A. sexdens* nests in Eucalyptus plantations can reach a high level and the nest distribution is generally concentrated (Camargo 2008; Souza et al. 2011). This may increase the effect of competition. Big colonies are also likely to have higher competitive advantages than small ones because their division of labor is more efficient and are thus able to handle a high rate of leaf fragments reaching their nests (Wilson 1980). In addition, the larger workers of big colonies are more resistant to adverse weather conditions such as high temperature (Bouchebti et al. 2015) or low humidity, can collect more vegetation biomass per capita and are more efficient for the defense of the colonies than the smaller workers of small young colonies (Fowler and Robinson 1979). Since small colonies are more likely to be inactive than large ones, they should also have less volume of fungus garden relative to their size and thus be more vulnerable to starvation than big colonies in case of prolonged periods of adverse weather conditions. This could be one of the explanations for the high rate of mortality observed in colonies in their early stage of development (Zanetti et al. 2014).

The number of hours favorable for foraging on the day preceding the survey did somewhat affect the probability of a nest being inactive: whatever their size, the colonies were less likely to be inactive when the number of hours favorable for foraging on the day preceding the survey was low. Therefore, a nest was more likely to be active if it had been active on the previous day and ants did not stop foraging because of a possible accumulation of vegetation biomass in their nest collected during the previous day. In fact, based on the area covered by loose soil above the nests, the nests in our survey should already count a high number of hambers containing fungus garden and thus be able to incorporate a large volume of vegetation. For example, a nest of 56 m², which according to the growth curve given by Grandeza et al. (1999) corresponds to a nest aged two years can count around 390 chambers containing fungus garden (Autori 1942; Swanson et al. 2019).

We found that the passage of the tractor over the nests had no impact on colonies' inactivity. This could be because *A. sexdens* nests are quite deep. Even in young colonies the depth of the chambers can range from 10 to 30 cm below the soil surface (Camargo et al. 2016). Therefore, the weight of the tractor and the pressure it exerted on the ground was probably not sufficient to cause significant damages to the nests. Moreover, the soils of our survey areas are characterized by a high content of clay and organic matter and stable and thus are less likely to lose their physical structure than other types of soil when submitted to a strong pressure (Bronick and Lal 2005).

Leaf-cutting ants show a high capacity to establish their nests in disturbed conditions, even in areas with high use of agricultural machinery. This explains why they reach a pest status in several agricultural crops (Robinson and Fowler 1982; Zanetti et al. 2014). Chemical control with poisoned granular baits is often the only method to control their population. Therefore, more knowledge on the factors influencing the period and duration of foraging inactivity in these ants could help to better target the time at which these baits can be applied so as not to harm non-target insects. In summary, knowledge about foraging inactivity in leaf-cutting ants is essential for the adoption of better management techniques and also to understand their intraspecific relationships.

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Normas do periódico Agricultural and Forest entomology (versão preliminar)

Artigo 2 - Is foraging activity in leaf-cutting ants more intense after periods of inactivity?

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Abstract: Leaf-cutting ants forage plant fragments at a circadian pace, but these patterns can be influenced by numerous factors, and may even cause foraging inactivation for several days. These insects have a high behavioral plasticity, so it is understandable to believe that ants present means of dealing with the scarcity of leaves after this period of inactivity. One of the main hypotheses is that the workers carry a greater amount of leaves after fasting to compensate for the period without offering leaves to the fungus. To test this hypothesis, 72 colonies of *Atta sexdens* were used, divided into different fasting periods (0, 10 and 20 days). The colonies were connected to a foraging arena within a BOD. Ten grams of leaves were offered in the foraging area and after 24 hours the remaining material was dried in an oven for 48 hours at 60°C and weighed on an analytical balance. The weight foraged by the ants is equal to the difference of the initial weight minus the final weight of the leaves. The mass lined by ants is not influenced by the fasting time, perhaps due to the processing capacity of the leaves. The number of chambers was restricted to only one in this work, that is, the ants carried enough material to the gardeners to incorporate the leaves regardless of the need for food. Leaf-cutting ants exhibit plasticity and adjust their behavior in relation to changes in the environment, but apparently the plasticity in foraging is independent of the internal factors of the colony. In addition, although adult ants feed on products derived from the symbiont fungus, about 90% of their energy and nutritional needs are derived from the consumption of sap from the plants at the time of their cutting. In this way, ants can adjust their needs by collecting leaves with more nutrients, since they have high nutritional flexibility, instead of spending more energy carrying more leaves that are unlikely to be assimilated by the colony. The inactivity of colonies for foraging is one of the biggest mysteries surrounding leaf-cutting ants. In this work it was possible to understand their behavior after their period. Since these ants are important herbivores in agricultural and natural forests, knowing that they no longer forage after the fasting period means that there is no increase in the damage caused by them.

Key words: insect behavior, foraging, behavioral flexibility, forest pests

INTRODUCTION

Ants are insects extremely diverse in their behavior, especially concerning their foraging activity (Viana-Bailez & Endringer, 2016). Some ants can forage for months to stock seeds that will be consumed during the winter period in which they do not forage due to extreme weather conditions (Jacquier et al., 2021). Others ants, such as leaf-cutting ants, feed on a symbiotic fungus which they cultivate inside their nests so that there is a need to maintain the supply of leaves throughout the year (Holldobler & Wilson, 2010).

Each ant species is characterized by a particular foraging pattern and follows a specific circadian rhythm which can be modified to adapt to changes in environmental conditions and food needs (Holldobler & Wilson, 1990). Temperature is one of the most important factors that regulate ants' foraging activity. For example, the leaf-cutting ant *Atta sexdens* forage at temperatures between 10° and 36°C and interrupt its foraging activity at temperatures outside this range (Fowler & Robinson, 1979; Giesel et al., 2013). Moreover, a rise of temperature can affect several components of foraging activity in leaf-cutting ants, e.g. the intensity of workers' flow on foraging trails (Ramirez-Olier et al., 2022), the speed of foraging workers (Tizón et al., 2014) or the mass of the leaf fragment they collect (Fowler & Robinson, 1979; Lima et al., 2006).

Other meteorological factors such as wind (Alma et al., 2016b, 2016a) and rain (Farji-Brener et al., 2018) have also been reported to cause momentary or long-term interruptions of foraging activity. However, even within the same location there is no common pattern of foraging activity in ant colonies of the same species. Therefore, factors other than meteorological conditions can probably influence ants’ foraging pattern. For example, one factor whose effect on ant foraging activity has been little studied so far is fasting. Leaf-cutting ants, such as *A. sexdens* are good models to test the influence of this factor at different temperatures. First, these ants are extremely sensitive to changes in temperature (ref). Second, they are known to carry a huge amount of leaves to their nests (Costa et al., 2018; Robinson & Fowler, 1982), so that any changes in their foraging behavior due to a fasting period should be easily detected. Finally, leaf-cutting ants are one of the most important herbivores in agroforest systems. Therefore, in order to predict the amount of damages they can cause in the context of global warming, any knowledge about the factors, such as fasting, that can affect their response to a change in ambient temperature is important.

The objective of this work was to evaluate the influence of a fasting period at different temperatures in leaf-cutting ants. Then, we assessed under laboratory conditions the amount of plant biomass foraged by the workers, the volume of colony fungus, the amount of waste accumulated and the rate of mortality at different temperatures and after different length of fasting periods. We aim at answering the following question: do leaf-cutting ant colonies take the risk of foraging in adverse temperature conditions after a long fasting period? do foraging ants carry more fragments to their nest after a long fasting period to compensate for the period of foraging inactivity?

The hypotheses are that the longer the fasting period, the higher nutritional needs of a colony. Therefore, the expectation is that a colony will collect more biomass after a fasting period and that the amount of biomass collected will increase with increasing length of the fasting period. Also, is expected that if the nutritional needs of the colony increase with the length of the fasting period ant foraging activity will be as high at extreme temperatures than at intermediate, more favorable, temperatures. It is believed that there will be an increase in the amount of waste and the number of dead ants during longer fasting periods and also in more inappropriate temperatures. This is due to the lack of fungus they use as main source of food for the entire colony, which results in greater accumulation of residues and an increase in the mortality of workers.

Material and methods

Study colonies

First of all, seventy-two semi-colonies of *A. sexdens* were transferred and reestablish, using part of the fungus with the ants (larvae, pupae, and workers) that were removed from different adult colonies (>3 years) of the laboratory Forest Pest Management Laboratory at the Universidade Federal de Lavras (Lavras, Minas Gerais – Brazil). The colonies were transferred to glass chambers (LxLxH: 20 x 20 x 2cm) which were placed on a plastic tray (60 x 40 x 9 cm), also chambers were totally occupied by fungus. They were kept in the laboratory in controlled conditions of temperature (24 ± 2° C) and humidity (RH 75 ± 10%). They were then supplied with fresh leaves (*Acalypha* sp.) and we waited a period of 7 days for the re-establishment of the colony, enough time for the reassembly of the fungus.

Experimental set-up and procedure

In the beginning of the experiments, the trays on which the colonies were placed were connected by a transparent PVC hose (diameter: 19 mm) to a foraging arena (circular plastic box of 500 ml). The foraging arenas were placed in a temperature-controlled BOD chamber (10, 16, 22, 28, 34 and 40° ± 2°C) in which fresh vegetation (*Acalypha* sp.) was offered at the beginning of every day during the 10-day experimental trials (Figure 4). The temperature inside the colony chambers was the same as that of the rearing room, i.e. 24°± 2°C. In addition, for each tested temperature, the colonies were put on a fast (without any supply of food) of 0, 10, or 20 days before the start of the experiment, during this fasting period, ants did not have access to the foraging arena (without leaves) inside the BOD. Twelve colonies were used for each combination of fast duration and temperature and four replicates were achieved for each combination of fast duration and temperature (6 temperatures x 3 fast durations x 4 replicates = 72 experimental trials for each colony).

At the start of each day during the experimental trials any vegetation remaining in the foraging arena (after 24 hours) was removed and replaced by fresh leaves. These fresh leaves were weighed on an analytical balance and then, after 24 hours, the unconsumed leaves were collected, dried for 48 hours in an oven at 60°C and weighed. To calculate the daily amount of leaves collected the mass of unconsumed leaves was subtracted to the initial fresh mass of leaves. These measured were done daily for 10 days.

The volume of fungus in each colony was measured at the start and at the end of each experimental trial by means of photography of the nest chambers. The area covered by the fungus (in cm2) was measured with the Free Software ImageJ and was then multiplied by 2 (height in centimeters of the glass chambers) to calculate the volume (in cm3). An assessment of ants' mortality at the end of each experimental trial was obtained by counting the number of dead ants in the waste. Also, the waste generated by the colonies was collected and weighed (mg) after being completely dried.

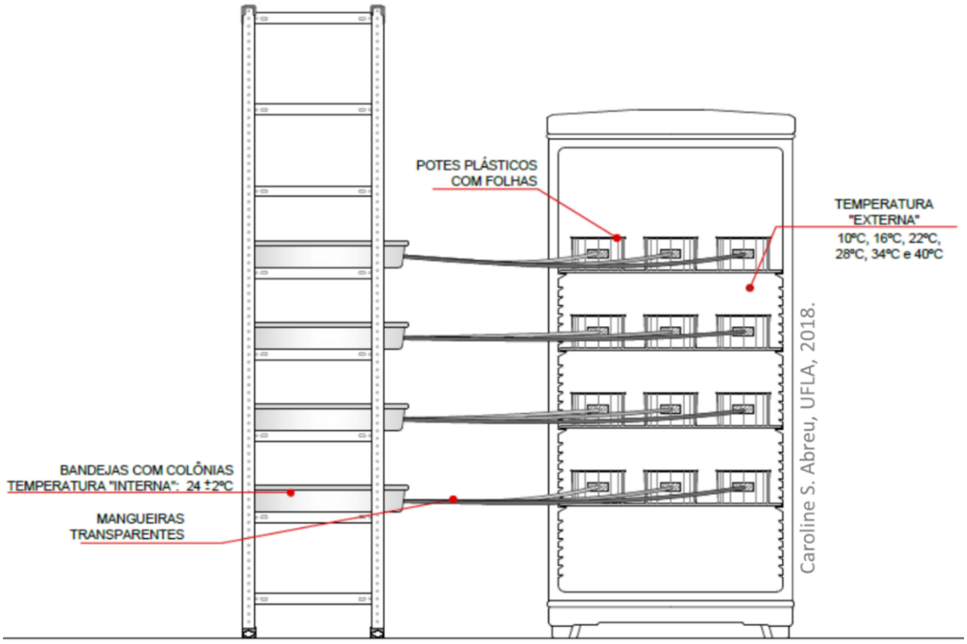

Figure 4. Experimental setup. The nests of *Atta sexdens* (Hymenoptera: Formicidae) were connected by a transparent hose to a foraging arena placed inside a thermostatic incubator (BOD).

Data analyses

The biomass foraged (mg) by ants was adjusted using a linear mixed model (LMM) in which temperature, fast duration, time (day), and all interactions between these variables were entered as independent variables. Both time and colony identity were added as random variables. We considered the interaction terms between independent variables because we expected that after fasting the vegetation biomass foraged by the colonies will be higher during the first days of the experiment, in other words, that workers will carry more fragments to compensate for the time without foraging, and the more so for long fast durations. In addition, temperature is strongly influenced by temperature (Giesel et al., 2013; Welch et al., 2020). Therefore, we expected that the variation over time in the amount of vegetation collected will be different across the temperatures tested and that the load of the fragments will be higher at temperatures more favorable for foraging, i.e., between 22 – 28°C (Giesel et al., 2013).

The volume of fungus (cm^3) and garbage generated by the colonies (mg) were modeled with linear mixed models (LMM), using colony identity as a random factor and temperature, fast duration, and the interaction between these variables as independent variables. The number of dead ants was modeled using a generalized linear mixed model (GLMM) with colony identity as a random factor and temperature, fast duration and the interaction between these variables as an independent variable. A posthoc test was used to compare the marginal means. The analyses were carried out using the statistical program R (R core team, 2023) and the R packages: lme4 (Bates et al., 2015); multcomp (Bretz et al., 2011), emmeans (Searle et al., 1980), ggplot (Wickam et al., 2022) and visreg (Breheny & Burchett, 2017).

RESULTS

The biomass of vegetation collected by ants was not influenced by fast duration. However, there was a significant interaction between time and temperature (Table 3). At 10°C the daily biomass collected by ants did not change over time whereas it increases at intermediate temperatures (16-28 °C) (Figure 5 and Figure 6).

The volume of fungus *Leucoagaricus gongylophorus* grown by ants varied with temperature (Table 5). At extreme temperatures, such as 10 and 34°C the volume of fungus was low (Figure 7). The total number of dead ants and amount of waste in the colonies was not influenced by either temperature or fast duration and there was not statistically significant interaction between these variables (Table 5).

Table 3. Influence of fast duration, temperature, time during the experiment and the interaction between these variables in the daily amount of vegetation biomass collected by ants, the volume of the fungus garden, the number of dead ants, and the weight of the waste in the studied *Atta sexdens* colonies (continued).

Models	**Factors**	**Df**	**Chisq**	**P**
Biomass collected	Fast duration	2	1.5109	0.46979
	Temperature	2	15.6923	**0.00039**
	Time	2	11.2314	**0.00364**
	Fast duration:Temperature	4	4.2882	0.36840
	Fast duration:Time	4	3.6609	0.45384
	Temperature:Time	4	12.5005	**0.01399**
	Fast duration:Temperature:Time	8	1.8160	0.98614

Table 4. Influence of fast duration, temperature, time during the experiment and the interaction between these variables in the daily amount of vegetation biomass collected by ants, the volume of the fungus garden, the number of dead ants, and the weight of the waste in the studied *Atta sexdens* colonies (conclusion).

Volume of Fungus garden	Fast duration	2	0.1597	0.92324
	Temperature	2	61.9414	**3.545e-14**
	Time	1	0.0826	0.7738
	Fast duration:Temperature	4	8.2998	0.08119
	Fast duration:Time	2	3.8205	0.14804
	Temperature:Time	2	1.4524	0.48374
	Fast duration:Temperature:Time	4	5.6662	0.22550
Number of dead ants	Fast duration	2	3.7225	0.155
	Temperature	2	0.4436	0.8011
	Fast duration:Temperature	4	3.095	0.5421
Weight of waste	Fast duration	1	1.5635	0.2112
	Temperature	2	5.7684	0.0559
	Fast duration:Temperature	2	1.2326	0.5399

p-values lower than the significance threshold of 0.05 are indicated in bold.

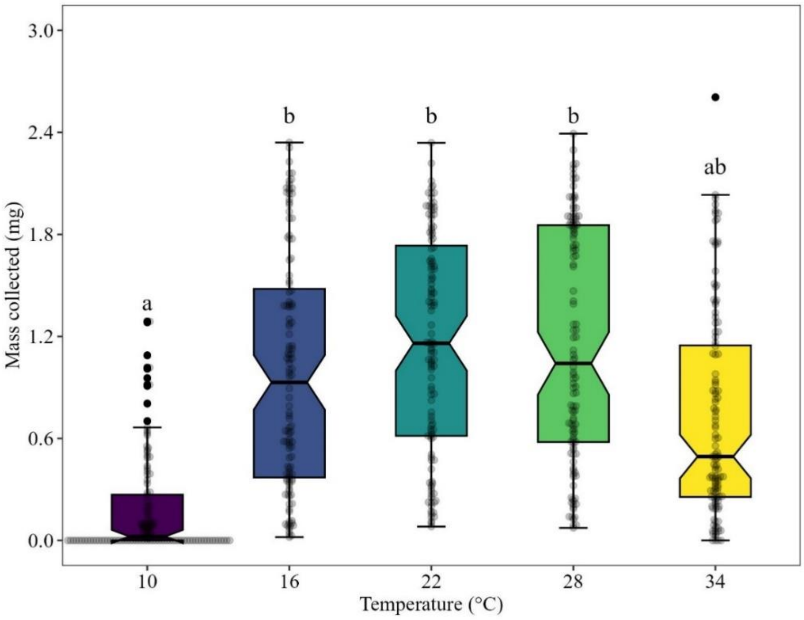

Figure 5. Mass (mg) of the leaf fragments transported by laden workers of *Atta sexdens* (Hymenoptera: Formicidae) at each tested temperature. The boxplots bearing the same letters indicate similar values at the 5% significance level (Marginal means posthoc test). The lines within the boxplots represent the median, the lower and upper boundaries of the boxes represent, respectively, the 25th and 75th percentiles, while the whiskers extend to the smallest and largest values within 1.5 box lengths. The open circles represent the outliers. The clearest dots represent a categorical scatter (beeswarm) which shows peaks in the data.

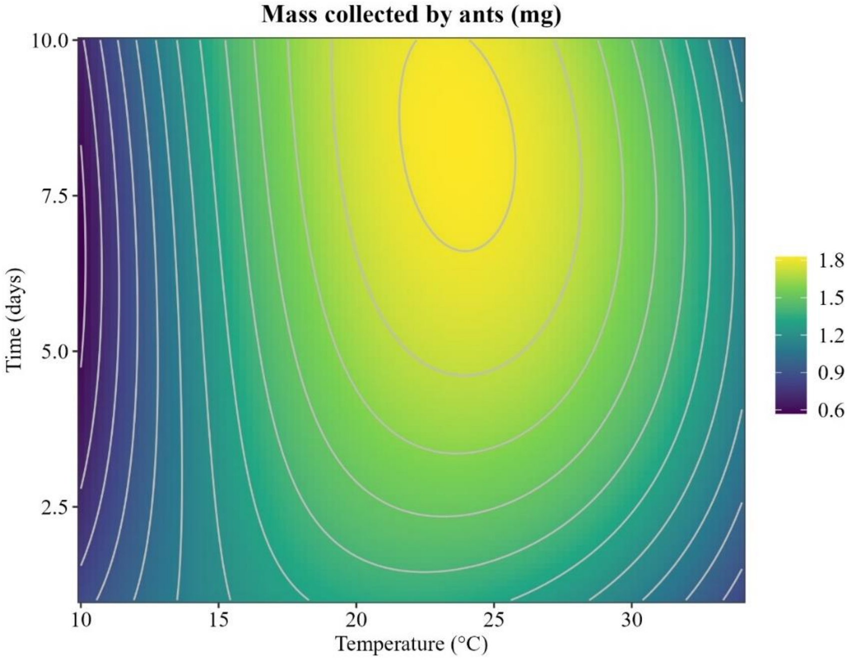

Figure 6. Daily amount of biomass collected (mg) by *Atta sexdens* (Hymenoptera: Formicidae) as a function of temperature (°C) and time (days).

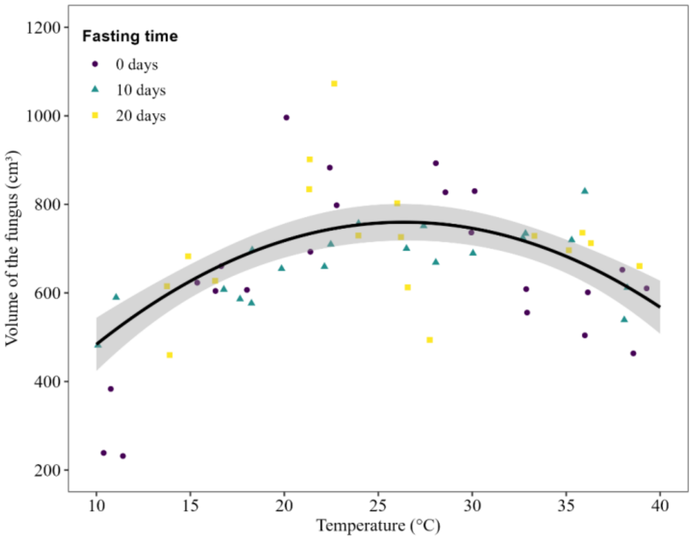

Figure 7. Volume of fungus (6cm³) grown by *Atta sexdens* (Hymenoptera: Formicidae) at each tested temperature and fast duration.

DISCUSSION

Colonies of leaf-cutting ants show different patterns of foraging behavior (Fowler & Robinson, 1979; Rockwood, 1976), including period of inactivity that can last for several days and during which no leaf material is brought back to the nest. In this work, we show that fast duration did not affect the daily biomass of vegetation collected by ants nor the volume of fungus, the mortality rate of the workers or the amount of waste deposition. However, both the amount of biomass collected and the volume of fungus at the end of the experiment were influenced by temperature. This is logical since these two elements are closely connected.

The reason why the amount vegetation biomass foraged by ants was not influenced by the duration of fasting may be connected to the processing capacity of the leaves by ants. In our experiment the number of chambers was restricted to only one. Therefore, foraging workers may carry enough material to the gardeners to incorporate the leaves regardless of the need for food. Leaf-cutting ants exhibit a high plasticity and they are able to adjust their behavior as a function of the changes occurring in the environment (Dussutour et al., 2008; Viana-Bailez & Endringer, 2016). However, the plasticity in foraging does not depend on internal factors of the colony, such as need of food. In addition, although leaf-cutting ant workers feed on the staphylae produced by the symbiotic fungus (Littledyke & Cherrett, 1976), about 90% of their energy and nutritional needs are derived from the consumption of plant sap that they ingest at the time of cutting (Quinlan & Cherrett, 1979; Bass & Cherrett, 1995). Therefore, since they show a high nutritional flexibility (Littledyke & Cherrett, 1976; Roces & Núñez, 1993; Shik et al., 2018a) ants may adjust their nutritional needs by collecting leaves with more nutrients rather than by spending more energy carrying leaves that are unlikely to be assimilated by the colony.

Ants carried more vegetation at intermediate temperatures. (16, 22, and 28°C) probably because of their behavioral and physiological limitations. Temperature has indeed a great influence on several components of leaf-cutting ant foraging behavior, as shown in *A. sexdens* (Fowler & Robinson, 1979; Giesel et al., 2013), *A. cephalotes* (Lewis et al., 1974), *A. capiguara* (Caldato et al., 2016), *Acromyrmex subterraneus molestans* (Ramirez-Olier et al., 2022), *Ac. subterraneus subterraneus* and *Acromyrmex crassispinus* (Nickele et al., 2016a, 2016b). These species show a high plasticity in their foraging behavior, which allows them to reach optimal foraging at more favorable temperatures and within their thermal limits (Tizón et al., 2014; Viana-Bailez & Endringer, 2016), which for *A. sexdens* is around 26°C (Caldato et al., 2016; Fowler & Robinson, 1979; Giesel et al., 2013). At these temperatures the rate of recruitment to the plants exploited is higher (Caldato et al., 2016; Giesel et al., 2013; Tizón et al., 2014), ants walk faster (Burd & Aranwela, 2003; Hurbert et al., 2008; Nelson et al., 2023) and carry larger leaf fragments to the nest (Giesel et al., 2013; Ramirez-Olier et al., 2022; Wirth et al., 1997) and consequently the rate of vegetation biomass arriving into the nest is higher. Also, when *A. sexdens* ants forage at temperatures near their thermal limits, i.e. at 10 and 36°C as in our experiment, they are more likely to suffer the harmful effects of extreme temperatures, such as dehydration and reduction of their metabolism (Lighton, 1994; Lima et al., 2022). In addition, ants will expend much more energy to perform the same foraging behavior at unfavorable temperatures, preventing the colony from being energetically efficient and taking advantage of the benefits of maximizing energetic returns per unit of foraging effort (Lighton, 1994; Lighton et al., 1987; Shik et al., 2019). Physiological limitations can also explain the fact that the daily amount of vegetation biomass collected by ants did not vary over time when ants were exposed to 10 and 32°C. In contrast, at intermediate temperatures they do not suffer from excessive energy expenditure (Shik et al., 2019) or dehydration (Lima et al., 2022). Hence, they can adjust the number of ants recruited to the exploited plants, the size and weight of the fragments they carry and, as a consequence, bring back to the nest a higher amount of vegetation biomass over time to fullfil the needs of the colony.

The volume of fungus was higher at intermediate temperatures and lower at extreme temperatures (10 and 36°C) because ants carried a greater number of leaf fragments into the nests when they encountered favorable weather conditions (Fowler & Robinson, 1979; Lima et al., 2006; Ramirez-Olier et al., 2022). As a consequence, the growth rate of the fungus was higher. The volume of fungus was similar for different fast durations at all tested temperatures, possibly because ants had a reserve of leaves that prevented the fungus from dying in the maximally 20-day fasting period, regardless of the external temperature. In fact, ants of the genus *Atta* can carry large amounts of leaves, from 11.68 to 500 kg of dry biomass per year (Caldato et al., 2016; Costa et al., 2008; Wirth et al., 2003). For this reason, despite the high rate of degradation and metabolization of fresh leaf material by the fungus garden (Moller et al., 2011), leaf fragments are progressively metabolized and transformed into fungal biomass (Moller et al., 2011; Shik et al., 2018b), making them have this reserve that prevents the fungus from quickly reducing its volume.

Mortality rate was not influenced by temperature and fast duration, because ants can continue to feed on the gongylidia produced by the fungus garden (Holldobler & Wilson, 2010; Mueller et al., 2005) since there was no decrease in its volume. Although leaf-cutting ants obtain their energy and nutrients mainly from plants' sap (Bass & Cherrett, 1995; Quinlan & Cherrett, 1979), they can feed on the products of the fungus if this resource is not available, which explains why the mortality rate remained the same regardless of the length of the fasting period and temperature. Moreover, the weight of the waste produced during the experiment was influenced neither by temperature nor the length of the fasting period. This can be explained by the fact that there was no decrease in the volume of the fungus and no increase in the rate of mortality due to lack of food caused by fasting or temperature. The waste is composed of parts of old fungus and carcasses of dead workers (Farji-Brener et al., 2016; Verza et al., 2017. Therefore, if there are enough workers to remove aggressive microorganisms from the colonies (Hart, 2002) as was the case for the colonies experiencing a fasting period, whatever its length, one can expect that there will be no differences in the amount of waste produced.

Why leaf-cutting ants show long period of foraging inactivity, in other words fasting period, is one of the biggest mysteries surrounding leaf-cutting ants. In this paper was possible to understand the ants’ behavior after its period. Since leaf-cutting ants are one of the most important herbivorous in agricultural systems and natural forests, knowing that they do not increase their foraging activity after a fasting period means that there will be no increase in the damages they can cause.

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Normas do periódico Physiological Entomology (versão preliminar)

Artigo 3 - Effect of body size on metabolic rates of workers of the polymorphic seed-harvesting ant *Messor barbarus* (Hymenoptera, Formicidae) exposed at different temperatures

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Normas do periódico The American Naturalist (versão preliminar)

Abstract: Caste polymorphism is an essential feature of eusocial insects, and of one of the most ecologically successful groups, ants. The great variation in body size observed in the caste of workers may also be associated with differences in their physiology that can be accessed by the metabolic rate (MR). Although the MRI value provides significant information, it is sometimes more interesting to express MRI per unit body mass (mass-specific MRI), as it allows comparing the MRI of animals of very different sizes under different conditions. The aim of our study was to evaluate the effect of temperature and body mass on metabolic rate in the polymorphic Mediterranean ant for seed harvesting *Messor barbarus.* In addition, we found that, depending on the size of the ant, mass-specific MRI is influenced differently by temperature: mass-specific MRI increases faster with increasing temperature in small ants than in large ants. However, the calculation of the Q10 value, a measure of ants' sensitivity to temperature changes, shows that large ants are more sensitive than small ants to a temperature change: whatever the initial temperature, large ants show a stronger response to a temperature change in terms of water loss and metabolism than small ants. These results show that ants of different sizes are characterized by different physiological strategies to cope with the increase in ambient temperature.

Key words: Polymorphism, Ants, Temperature.

INTRODUCTION

Caste polymorphism is an essential characteristic of eusocial insects and of one of the most ecologically successful group of these insects, i.e ants. It is generally related to the division of labor observed within colonies (Wilson 1980). For example, in most species of ants showing worker size polymorphism, medium-sized to large workers are mostly seen outside the nest and participate in foraging activities while small workers stay inside the nest to care for brood and the queen (Wilson 1980; Lighton 1994). The large variation in body sizes observed in the worker caste can also be associated with differences in their physiology (Robinson 2009), e.g. their thermal preference or thermo-tolerance.

It has been shown repeatedly in polymorphic ants that, within the same colony, the critical thermal maximum (CTmax, the temperature at which knockdown or the onset of muscle spasms is observed in an insect) is higher in large workers than in small workers (*Atta sexdens rubropilosa:* Ribeiro et al. 2012; *A. capiguara* and *A. laevigata*: Bouchebti et al. 2015; *A. colombica*: Welch et al. 2020; *Messor ebeninus* and *M. arenatius*: O'Donnell et al. 2020; *A. cephalotes*: Baldier and O'Donnell 2020). Yet, one must be cautious with the interpretation of CTmax because it is a measure of insect mortality at extreme temperatures and is thus only a rough measure of thermal tolerance. Moreover, some methodological issues arise because the evaluation of CTmax may vary according to the procedure used to test the ants, notably the duration of the experiment and the heating rate that is used to reach the critical temperature (Ribeiro et al. 2012). A much better approach is the measure of the thermal reaction norm of individual ants, i.e. the pattern of variation of their response to increasing temperatures. This response can be assessed by the metabolic rate (MR), which corresponds to the amount of energy an individual needs to keep its body functioning (Shik et al. 2019).

Measurements of MR in species of ants showing worker size variation within colony have already been performed in *Anoplolepis steinergroeveri* (Chown et al. 2007), *Atta colombica* (Chown et al. 2007), *Camponotus spp.* (Chown et al. 2007; Waters 2014), *Eciton hamatum* (Chown et al. 2007), *Formica rufa* (Chown et al. 2007), *Messor spp.* (Chown et al. 2007), *Formica rufa* (Perl and Niven, 2018) and *Aphaenogaster iberica* (Shik et al. 2019). In all of these studies, MR has been shown to scale allometrically with body size. However, the exponent of the equation describing the allometric relationship between MR and body size varies a lot between species. For example, in *Messor pargandei* the value of the slope is 0.61, whereas in *M. capensis* it is 1.28 (Chown et al. 2007) and in *Formica rufa* (Perl and Niven 2018) it lies between 0.4 and 0.59.

Although the value of MR gives meaningful information, it is sometimes more interesting to express the MR per unit body mass (mass-specific MR) because it allows to compare the MR of animals of very different sizes in different conditions. In this case, a general rule in insects (Lighton et al. 1987; Full and Tu 1991) as in other animals (Schmidt-Nielsen 1984), is that the mass-specific MR decreases with increasing body mass, i.e. small animals consume more energy per unit body mass than large animals. Mass-specific MR is a good way to measure differences in thermal physiology of ants originating from different environments. For example, it allows to measure the reaction norm of ants collected along an elevation gradient to increasing temperatures (Shik et al. 2019). The energetic cost of maintaining its body at different temperatures can then be related to the actual range of worker size observed within colonies and to the division of labor between individuals of different sizes observed within colonies. Hence, due to the negative relationship between mass-specific MR and body mass, one can hypothesize that large workers should consume less energy per unit body mass than small workers and thus that they should be overrepresented in the foraging worker force. Moreover, because of their lower body surface area to body mass ratio, large workers should have a lower heating rate and be more tolerant to high temperatures. Consequently, ant colonies with a larger range of foraging worker size should have a competitive advantage because they should be able to extend their foraging activity across a larger range of temperatures.

The objective of our study was to assess the effect of both temperature and body mass in respiratory water loss and mass-specific MR in the Mediterranean polymorphic seed harvesting ant *Messor barbarus*. If the relationship between mass-specific MR and body size generally observed in animals holds for this species, we expect that the regression curves of mass-specific MR against temperature will have a higher elevation for small ants than for big ants. In addition, if small ants are more sensitive to increasing temperature due to the higher body surface area to body mass ratio, we expect that the proportion of water lost and their mass-specific MR will increase more rapidly with increasing temperatures than that of large ants. If this is not the case, this could suggest that some compensatory mechanisms exist in small workers, e.g. the activation of heat shock protein (Gehring and Wehner 1995) or morphological adaptation such as a thicker cuticle, allowing them to forage across a larger range of temperatures than would be allowed if their upper thermal limit depended only on their body surface/mass ratio.

METHODOLOGY

Study colonies

Three colonies of *Messor barbarus* were collected in Saint-Hyppolyte, near the city of Perpignan, on the French Mediterranean Coast. Each colony was kept in the laboratory in a plastic box which contained several test tubes wrapped with white paper and partially filled with water retained by a cotton plug in which the ants could establish their nest. The colonies were kept in a room at a temperature of 25°C with 50% relative humidity. They had access to water *ad libitum* and were fed daily with a mixture of seeds of various species.

Metabolic rate (MR) measurements

The MR of individual workers was measured at five different temperatures (15, 20, 25, 30, and 35ºC) with a high resolution respirometric system (Sable Systems Europe GmbH, Berlin, Germany) equipped with a nondispersive infrared CO_2 analyzer (LI-COR 850, LI-COR, Lincoln, NB, USA, lower limit detection: 1.5ppm). During the trials ants were placed individually in seven cylindrical glass respirometry chambers (diameter: 2cm, length: 7cm, effective air volume: 14.14cm^3) housed in an insulated box (PELT-DROP-IN) whose temperature was regulated by a Peltier controller (PELT-5, control stability: 0.01-0.20°C). An eighth chamber remained empty and was used for baselining. The inside of the box was lighted by LEDs (Cineroid L10-BC) and a webcam (Logitech Brio 4k ultra-HD) filmed the ants inside the chambers to measure their activity. The temperature inside the insulated box was continuously recorded using a thermistor cable.

We performed constant volume respirometry (Lighton and Halsey 2011). In each trial ants were kept in airproof respirometric chambers for a total of three hours. At 30min intervals the air of each chamber was flushed for 200s and replaced by fresh air scrubbed of H_2O and CO_2 through the passage in a drierite/ascarite/drierite column. The flow rate was set at 50 ml/min by a subsampler pump (SS-4) that was regulated by an Alicat Scientific MC Series valve (MFCV-31) connected to a mass flow control unit (MFC-2). The air in the chambers first passed through a column of magnesium perchlorate (Cl_2MgO_8) to remove the water vapor due to the insects' respiration and then was directed to the CO_2 analyzer to measure the CO_2 accumulated in the chambers (expressed in µl CO_2 hr^{-1}). A multiplexer (RM-8) programmed by a software provided by Sable Systems (SW-EXPEDATA-P) operating through an interface unit (UI-3) allowed to flush the air sequentially from the eight respirometric chambers. The MR measurements were obtained automatically by the software through the integration of the area below the CO_2 peaks of the excurrent air flow. The CO_2 values measured from the empty chamber were subtracted from the values obtained from the chambers containing ants to correct for possible leaks. The MR values measured for each ant were averaged only over the last five

30-min periods of the trials. In fact, since the air was not scrubbed of CO_2 at the moment ants were installed in the chambers, the MR measurements of the first 30min period were excluded from the analysis. The CO_2 analyzer was calibrated every three respirometric trials, zeroing it with N_2 gas and then spanning it with a gas of known CO_2 concentration (5,000 p.p.m. CO_2 in N_2 ± 1%).

Ants from three different colonies of *M. barbarus* were used in the trials. For each experiment seven ants were captured directly outside their nest, in the foraging box of the colonies. The size of the ants was assessed visually and categorized in three size classes as small, medium or large. Ants were then weighed a first time with an analytical balance (Metler Toledo MS105: accuracy: 0.01mg) to get their fresh mass. This fresh mass was used to calculate the mass-specific MR ($\mu lCO_2.h^{-1}.mg^{-1}$), i.e. the value of MR obtained from the SW-EXPEDATA-P software divided by the fresh mass of the ants. The ants were then placed in the respirometric chambers. To avoid any perturbation due to pheromone deposits, the respirometric chambers were washed with a neutral detergent and dried with paper towel every time ants from a different colony were used. All ants were immediately weighed at the end of the experiment to assess the mass they lost by evaporation during the experiment. In total, the MR of 144 ants (48 of each size class) for each of the five temperatures tested was measured.

Ant activity

Ant locomotor activity was measured for each ant during the respirometric trials by the number of times ants crossed an imaginary line in the middle of the respirometric chambers. As for the MR, the activity score was averaged over the last five 30-min periods of each respirometric trials

Data analyses

From a theoretical point of view one should expect that the proportion of water lost by ants should be proportional to their body surface area. Since body surface area increases as the square of body length whereas body mass increases as the cube, one should expect that the proportion of water lost by ants should scale allometrically with body mass with a 2/3 exponent. The allometric equation can thus be linearized as: log(proportion of water lost) $\approx$ -0.66 log(body mass). Consequently, the effect of body mass (mg) and temperature (°C) on the proportion of water lost in ants was tested with a GLM with body mass and proportion of water lost expressed on a log scale. In addition, to account for the fact that the proportion of water lost with increasing temperatures may vary differently with body size, e.g. because of different thickness of the cuticle (Peeters et al. 2017), we also considered the interactions between body mass and temperature in the statistical model.

To compare the thermal sensitivity of ants of different sizes as regards to the loss of water we calculated the Q_{10} value, which is commonly used in physiology to study the reaction norm of animals across a thermal gradient (Shik et al., 1999). Here, the Q_{10} corresponds to the rate of change in the proportion of water loss across 10ºC temperature intervals. To calculate the instantaneous Q_{10}, we used the derivative of the equation of the statistical model above which relates the value of the proportion of water lost by ants as a function of their body mass and temperature (Vogt and Appel 1999; Lighton 2018; Shik et al. 2019).

According to the literature on animal physiology, the mass-specific MR of small animals is higher than that of big animals and scales allometrically with their body mass with a negative exponent varying between -0.25 and -0.33 (Schmid-Nielsen, 1984). This equation was linearized so that the effect of body mass (mg) and temperature (°C) on the mass-specific MR of ants in our experiment was tested with a GLM with both mass-specific MR and body mass expressed on a log scale. Since mass-specific MR may decrease non-linearly with increasing temperature (Shik et al., 2019), we entered a quadratic term for temperature in the model. Finally, since the effect of temperature on mass-specific MR may vary in ants of different sizes, we also considered the interaction between body mass and temperature in the statistical model. To compare the thermal sensitivity of ants of different sizes as regards to the mass-specific MR we calculated the Q_{10} value, i.e. the rate of change of the mass-specific MR across 10ºC temperature intervals.

Finally, the influence of temperature and body mass on ants' activity was modeled by a GLM with a quasi-Poisson error distribution. The interaction between body mass and temperature was included in the model because we hypothesized that activity may vary differently with temperature in ants of different sizes (Hurbert et al. 2008; Welch et al. 2020). To account for the fact that ant activity could reach a plateau at high temperatures we entered a quadratic term for temperature in the model.

The value of body mass used in the models was always the fresh mass of the ants. Moreover, temperature was considered as a continuous variable in all models. All data analyses were run and figures generated with R (4.2.0) run under RStudio (1.4).

RESULTS

Figure 1 shows the distribution of the fresh mass of the workers used in the experiment. One can distinguish three size classes: small (≤ 5mg), medium (>5mg and ≤ 15mg) and large

(>15mg). The mass of the smallest worker was 0.70mg while that of the largest was 41.50mg; the median mass of each size class was 2.30, 9.40, 26.10 for small, medium and large ants respectively.

There was a significant effect of body mass and temperature on the proportion of water lost (Table 5). The proportion of water lost increased with increasing temperature and decreased allometrically with increasing body mass with an exponent comprised in the interval [-0.844 - 0.596] (Figure 9a, Table 5), which thus included the theoretical value of 0.66 corresponding to the ratio of body surface area to body mass. Moreover, there was a significant interaction effect between temperature and body mass (Table 5): the proportion of water lost increases more rapidly with increasing temperature in small ants than in medium or large ants (Figure 9b). The sensitivity of ants to a change in temperature increases with increasing body mass (Figure 9c): the Q_{10} value of large ants was higher than that of medium ants which was itself higher than that of small ants (Figure 9d).

Table 5. Results of the statistical models used to investigate the effect of fresh body mass and temperature on the proportion of water lost, the mass-specific MR and the ants' activity.

Models	Factors	β Coeff [$CI_{95\%}$]	Sum Sq	F value	R2 adj.	*P*
log(proportion of water lost)	log(Body mass)	-0.720 [-0.844 -0.596]	133.977	579.849	0.59	2.2e-16
	Temperature	0.028 [0.017 0.039]	106.436	460.649	0.59	2.2e-16
	log(Body mass) x Temperature	0.013 [0.008 0.018]	6.176	26.728	0.59	3.049e-7
log (mass specifc MR)	Log(body mass)	-0.650 [-0.716 -0.584]	167.966	2564.146	0.85	2.2e-16
	Temperature	0.090 [0.067 0.114]	88.495	1350.953	0.85	2.2e-16
	Temperature^2	-0.0011 [-0.0016 -0.0007]	1.735	26.488	0.85	3.439e-7
	log(body mass) x Temperature	0.008 [0.005 0.010]	2.362	36.060	0.85	3.057-09

p-values lower than the significance threshold of 0.05 are indicated in bold. Df=1 for all models and factors

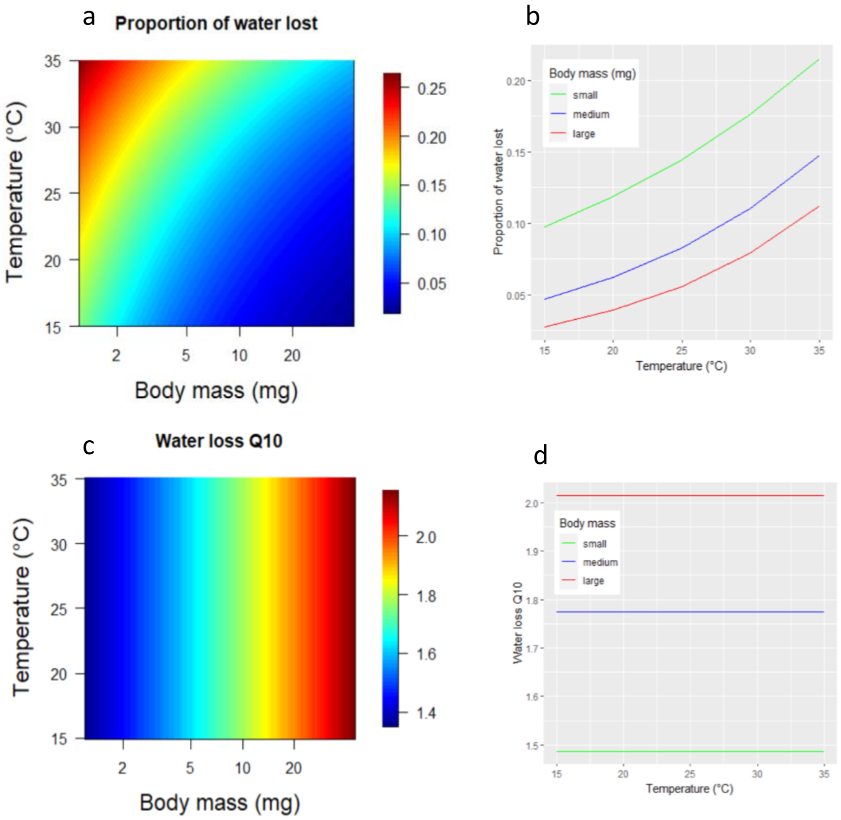

Figure 8. Proportion of water lost in *Messor barbarus* ants. (a) Proportion of water lost as a function of fresh body mass and temperature. (b) Proportion of water lost as a function of temperature for the median mass of each worker size class. (c) Instantaneous Q_{10} values for the proportion of water lost as a function

of body mass and temperature. (d) Instantaneous Q_{10} values as a function of temperature for the median mass of each worker size class.

The mass-specific MR increased with increasing temperature and decreased allometrically with increasing body mass with an exponent comprised in the interval [-0.716 - 0.584] (Table 4). In addition, the mass-specific MR varied differently with temperature for ants of different sizes (Table 4: interaction between temperature and body mass). The mass-specific MR of small ants increased more rapidly with increasing temperature than that of medium or large ants (Figure 10b). The instantaneous mass-specific MR Q_{10} decreased with increasing temperatures and, whatever the temperature, was higher for large ants than for medium or small ants (10c). Moreover, the mass-specific Q_{10} decreased more abruptly with increasing temperatures for large ants than for small ants (Figure 10d) showing that, regarding the energy required to maintain their body functioning, large ants were more sensitive to a change in temperature than small ants.

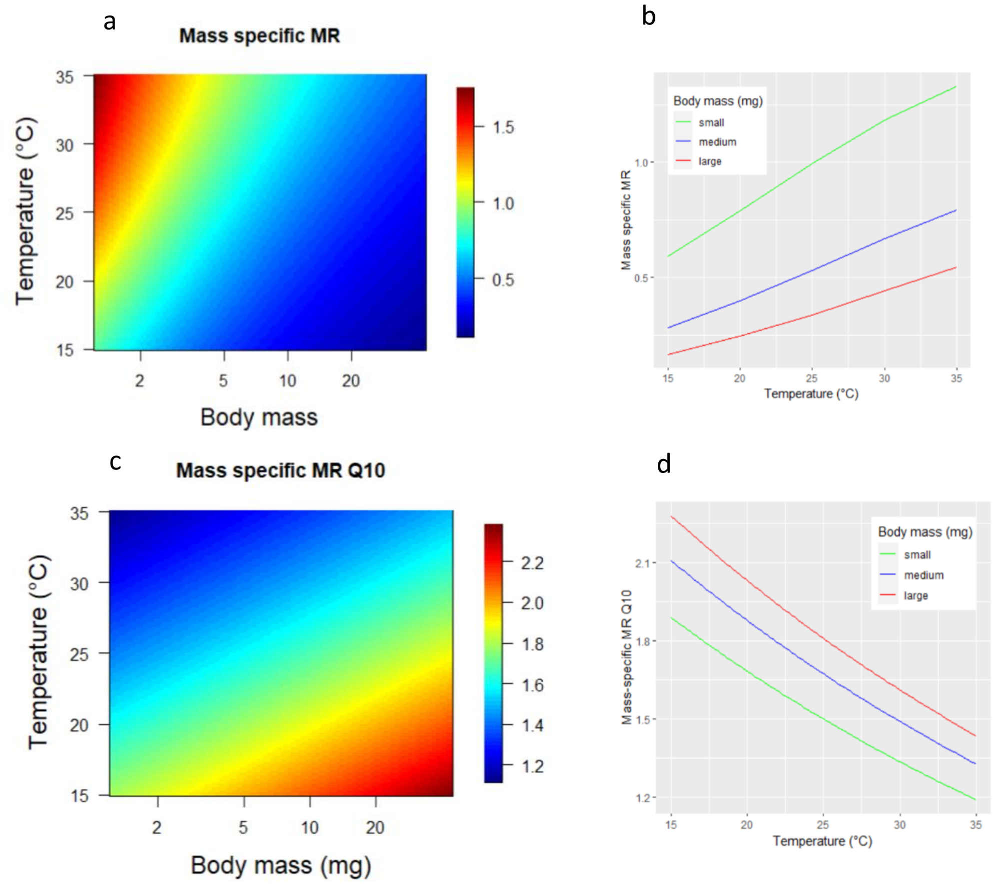

Figure 9. Mass-specific metabolic rate (log (µCO2.mg^{-1}.h^{-1} of *Messor barbarus* ants. (a) Mass specifc MR as a function of fresh body mass and temperature. (b) Mass-specific MR as a function of temperature for the median mass of each worker size class (c) Instantaneous Q_{10} values of mass specific MR as a function temperature and body mass. (d) Instantaneous Q_{10} values as a function of temperature for the median mass of each worker size class.

The activity of ants did not depend on their body mass (Table 4, Figure 4a, but note that this is a partial result since activity has not yet been measured on all ants). However, it increased non linearly in the same way with increasing temperatures for ants of all size classes (Table 4, Figure 11ab).

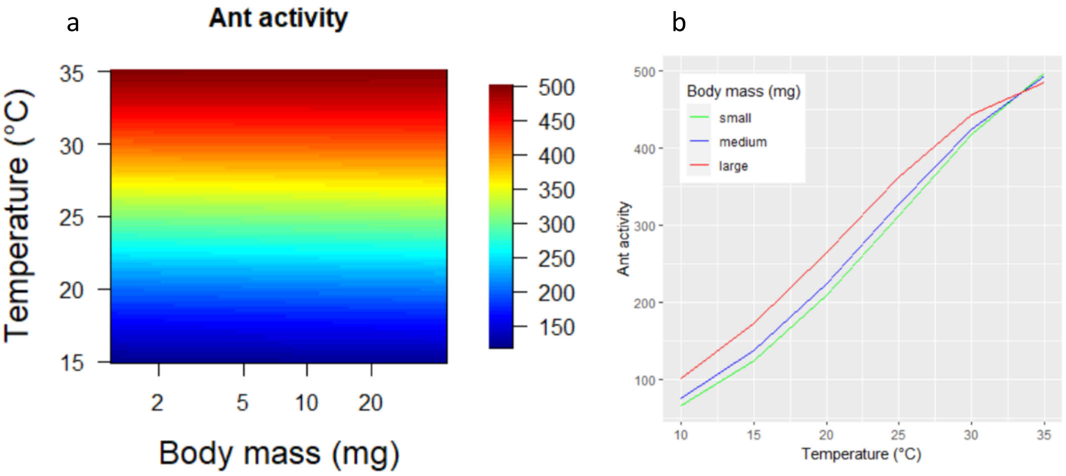

Figure 10. Ant activity as measured by the number of times ants crossed a line in the middle of the respirometric chamber. (a) Ant activity as a function of fresh body mass and temperature. (b) Ant activity as a function of fresh body mass for the median mass of each worker size class. N= 14, 14, 13, 13, 7 ants for 15, 20, 25, 30, 35°C respectively.

DISCUSSION

Caste polymorphism is one of the main characteristics of social insects and a feature that is probably responsible for their ecological success in a variety of biomes and ecosystems (Trible and Kronauer 2017). In ants for example, size variation within the worker caste has been shown to be important to keep a high level of foraging efficiency (Wilson 1983; Waser 1998; Reyes-Lopez and Fernandez-Hager 2001; Arnan et al. 2011; Constantino et al. 2021; Ramirez-Olier et al. 2022). In this study we found that large ants have a lower rate of water loss than small ants and, consequently, that they are more tolerant to desiccation, one of the most important stress factors in animals. Moreover, we found that, depending on ant size, mass-specific MR is differently influenced by temperature: mass-specific MR increases more rapidly with increasing temperatures in small ants than in large ants. However, calculation of the Q_{10} value, a measure of the sensitivity of ants to changes in temperature, shows that large ants are more sensitive than small ants to a change in temperature: whatever the initial temperature, large ants show a stronger response to a change in temperature in terms of water loss and metabolism than small ants. These results show that ants of different sizes are characterized by different physiological strategies to cope with an increase in ambient temperature.

Our expectation was that large ants would lose proportionally less water with increasing temperature than small ones because of their smaller body surface area to body mass ratio (Edney, 1977; Chwn et al. 2011). Our results show that this was indeed the case: whatever the temperature, small ants had a higher rate of desiccation than large ants. In addition, we found that the rate of desiccation of small ants increases more rapidly with increasing temperatures than that of large ants. The differences in the rate of desiccation of ants of different sizes for increasing temperatures may be explained by differences in their morphology or physiology. For example, differences in ventilation rate (Lighton 1990, 1992; Perl and Niven 2018), the amount of epicuticular lipids (Hood and Tschinkel 1990), and the thickness, pilosity or abrasion level of the cuticle may affect its permeability and thus the rate at which ants lose water (Lighton and Feener 1989; Johnson 2000; Ajayi et al. 2020; Buxton et al. 2021). For example, in the ant *Solenopsis* spp. the permeability of the cuticle varies with body size so that big ants have a lower rate of desiccation than small ants (Ajayi et al. 2020). In addition, the properties of the cuticle may also be affected and in particular the way its permeability changes with ambient temperature (Mead-Briggs 1956; Lighton and Feener 1989; Schilman et al. 2007). In *M. barbarus* large ants are not an enlarged copy of small ones because some of their body parts (e.g. their head) increases allometrically with their body size (Bernadou et al. 2016). Therefore, large workers of *M. barbarus* may be more tolerant to high temperatures and more prepared to cope with heat than small ants not only because of their size but also because of their larger head and a thicker cuticle. Such a higher tolerance to high temperatures has been documented in other polymorphic ants (*Cataglyphis velox:* Cerdá and Retana 1997; *C. cursor:* Clémencet et al. 2010*; Atta cephalotes*: Baudier and O'Donnell 2020; *A. sexdens:* Ribeiro et al. 2012; *A. capiguara* and *A. laevigata*: (Bouchebti et al. 2015), *Messor ebeninus* and *M. arenarius*: Baudier and O'Donnell 2020). Overall therefore, our results are consistent with those observed in large workers of other species, whether it is for their lower rate of desiccation (Lighton and Feener 1989; Bujan et al. 2016; Perl and Niven 2018) or for their higher thermal tolerance (Kaspari et al. 2015; Roeder et al. 2021).

As shown in other studies in ants (Waters 2014; Colinet et al. 2015; Perl and Niven 2018; Shik et al. 2019; Packard 2020), we found that, whatever the size of the ants, mas-specific MR was positively correlated with temperature. Moreover, as expected by the data from the literature, mass-specific MR was positively correlated with body size at all temperatures. This may explain why in ant species showing a high size variation of the worker caste, medium and large ants are overrepresented on foraging trails compared to small ants (Wilson 1980; Heredia and Detrain 2005; Ramirez-Olier et al. 2022), insofar as they need less energy per unit body mass to keep their body functioning. In the same time, this allows to increase foraging efficiency since they can carry bigger and heavier loads back to the nest.

An allometric relationship between mass-specific MR and body size has been reported in a lot of animals, including ants (Riveros and Enquist 2011; Waters 2014; Perl and Niven 2018; Packard 2020). The allometric exponent of the mass-specific MR, calculated across a variety of animal ranging in size between insects to elephants has been found to be around -0.25 (Schmidt-Nielsen 1984) the value expected under the nutrient supply network model of the metabolic theory of ecology (Chown et al. 2007). The alternative theory, that of the cell size model which states that the metabolic rate is a by-product of the way in which body size changes, predicts values for the allometric exponent between 0 and -0.33 (Chown et al. 2007). The value we found, i.e. in between -0.584 and -0.716, was much lower and thus differed from the values predicted by both theories. However, it is compatible with the value found by Perl & Niven (2018) in the red wood ant *Formica rufa*, i.e. between -0.41 and -0.60. Although still much lower than the value found by the latter authors, the lower value of allometric exponent found in our study is consistent with the fact that the mass-specific metabolic rate scaling exponents are generally lower in intraspecific studies than in interspecific studies (Lighton, 1989; Perl & Niven, 2018). One explanation for the discrepancy in allometric exponent values reported in the literature and that found in our study may lie in the fact that our statistical model does not yet control for the confounding effect of ant activity. In fact, metabolic rate increases with increasing activity. Therefore, if ants of different sizes differ in their level of locomotor activity inside the chambers during the respirometric trials, e.g. with large ants being less active than small ants or small ants being more active than large ants (which however does not seem to be the trend shown by our partial analysis of activity level), the value obtained for the scaling exponent may be lower than that expected by the two theories exposed above and closer to the value we found.

The reverse pattern as that observed for the mass-specific MR for increasing temperatures was observed for the sensitivity of ants to change in temperatures: although the mass-specific MR of large ants was lower than that of small ants at all tested temperatures, their sensitivity to a change in temperature, calculated as an instantaneous Q_{10} value, was higher. Moreover, whatever the size of the workers, their sensitivity to a change in temperatures decreased with increasing temperatures, a result also found in *Aphaenogaster senilis* colonies collected above 1000m elevation in southern Spain (Shik et al. 2019) or in *Solenopsis invicta*

(VOGT; APPEL, 1999), but at contrast with that found in the African ant *Camponotus fulvopilosus* for which the Q_{10} increases for temperature above 25°C (Lighton, 1989). As underlined by Lighton (1989), it may be advantageous for ants to forage at temperatures where their metabolic rate is not subject to rapid increase with changing temperature, which are associated to higher foraging costs for the colony and to higher variation in foraging performances. In the case of *M. barbarus* this means that the range of favorable temperatures for foraging should vary with worker size, with workers of increasing size leaving the nest to forage as the ambient temperature increases. One can thus predict that in polymorphic species such as leaf-cutting ants of the genus *Atta* or seed harvesting ants of the genus *Messor*, the proportion of large ants on foraging trails should increase at high ambient temperatures. In addition, our calculation of Q_{10} allows us to conclude that the critical event for ants, i.e. the moment at which energy consumption increases the most, is when they leave their underground nest, that remains at a low and relatively constant temperature, to emerge in the hot outdoor environment.

Finding out how body size influences the thermal physiology of ants is essential for a better understanding of the foraging pattern of these insects, one of the most abundant and ecologically successful organisms in the world. By coupling the study of the effect of body size and temperature on energetic metabolism, our study allows to shed light on the thermal physiology of polymorphic ants and on its consequences for the size-related division of labor observed in foraging tasks.

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3 CONCLUSÕES GERAIS

As formigas apresnetam comportamentos extremamente complexos que dependem do ambiente. O conhecimento sobre a proporação de colonias inativas baseado em fatores climáticos e uso de maquinário agrícola poderia ajudar a direcionar melhor o momento em que essas iscas podem ser aplicadas para não prejudicar insetos não-alvo. Em síntese, o conhecimento da inatividade de forrageamento em formigas cortadeiras é essencial para a adoção de melhores técnicas de manejo e para o entendimento de suas relações intraespecíficas.

As formigas cortadeiras apresentam um período de inatividade de forrageamento que é um dos maiores mistérios em torno das formigas cortadeiras. Neste trabalho foi possível compreender o comportamento das formigas após o seu período. Como as formigas cortadeiras são um dos herbívoros mais importantes em sistemas agrícolas e florestas naturais, saber que elas não aumentam sua atividade de forrageamento após um período de jejum significa que não haverá aumento nos danos que podem causar.

O conhecimento sobre os fatores fisiológicos que determinam as mudanças de comportamento é essencial para a adoção de táticas mais precisas para o manejo de formigas cortadeiras. Descobrir como o tamanho do corpo influencia a fisiologia térmica das formigas é essencial para uma melhor compreensão do padrão de forrageamento desses insetos, um dos organismos mais abundantes e ecologicamente bem-sucedidos do mundo. Ao acoplar o estudo do efeito do tamanho e da temperatura corporal no metabolismo energético, nosso estudo permite lançar luz sobre a fisiologia térmica de formigas polimórficas e suas consequências para a divisão do trabalho relacionada ao tamanho observada em tarefas de forrageamento.