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Seasonal Effects of Abiotic Factors and Nutrient Sources on the Feeding Activity of the Harvester Ant Species *Messor semirufus* (André, 1883)

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ABSTRACT

This study aims to investigate seasonal effects of abiotic factors and nutrient sources on the feeding activity of the harvester ant species *Messor semirufus* (André, 1883). Activation rhythms and feeding regimes were observed for *M. semirufus* workers in three seasons, spring, summer and autumn, for one year. Abiotic factor measurements were made every month and the recorded values were entered into follow-up tables. It was determined which plant seeds *M. semirufus* workers preferred from the plants naturally growing in the observed nest habitat. In addition, different food seeds that are not naturally found in the nest habitat of *M. semirufus* were placed near the nest and seed preferences among these were observed and noted. Activation of *M. semirufus* workers was at its maximum level when the soil temperature reached 30°C. Two plant families that *M. semirufus* prefers the most in the natural environment are the Poaceae (Grains) and Asteraceae (Daisy) seeds. On the other hand, in experimental studies on seed preferences, 12 types of seeds with different nutritional values, differing from the plants in the natural environment, were left around the *M. semirufus* nest. Of these, wheat, which contains 61% carbohydrates, was the most preferred seed by workers. The workers' second choice was flaxseed, which contains more oil. The later preferred seeds are oat, spinach seed, sesame, millet, chickpea seeds. Safflower, sunflower, rapeseed, hemp and broad bean are among the seeds that are not preferred.

Keywords: Harvester ants; Seasonal activity; Seed preference; Nutrient source; Abiotic factors.

Abiyotik Faktörlerin ve Besin Kaynaklarının Hasatçı Karınca *Messor semirufus* (André, 1883)'un Beslenme Aktivitesi Üzerindeki Mevsimsel Etkileri

ÖZ

Bu çalışmada abiyotik faktörlerin ve besin kaynaklarının bir hasatçı karınca türü olan *Messor semirufus* (André, 1883)'un beslenme aktivitesi üzerindeki mevsimsel etkileri incelenmiştir. Bir yıl boyunca *M. semirufus* için ilkbahar, yaz ve sonbahar aylarında olmak üzere 3 mevsim aktivasyon ritimleri ve beslenme rejimleri gözlemlenmiştir. Her ay abiyotik faktör ölçümleri yapılarak kaydedilen değerler takip çizelgelerine kaydedilmiştir. Gözlem yapılan yuva habitatında doğal olarak yetişen bitkilerden, *M. semirufus* türünün hangi bitki tohumlarını tercih ettiği saptanmıştır. Ayrıca, *M. semirufus* türünün yuva habitatında doğal olarak bulunmayan farklı besin tohumları da yuva yakınına konularak bunlar arasından tohum tercihleri gözlenerek not edilmiştir. *M. semirufus* işçilerinin toprak sıcaklığı 30°C'yi bulduğunda aktivasyonlarının maksimum düzeyde olduğu gözlenmiştir. *M. semirufus*'un doğal ortamda en çok tercih ettiği iki familya Poaceae (buğdaygiller) ve Asteraceae (papatyagiller) familyalarıdır. Tohum tercihlerine yönelik deneysel çalışmalarda, *M. semirufus* yuvası çevresine doğal ortamdaki bitkilerden ayrı, farklı besin değerlerinde 12

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çeşit tohum bırakılmıştır. Bunlardan % 61 oranında karbonhidrat içeren buğday en çok tercih edilen olmuştur. İkinci sırada içeriği daha çok yağ olan keten tohumu olmuştur. Yulaf, ıspanak tohumu, susam, darı, nohut tohumları öncelikli türlerden sonra yuvaya taşınılan türlerdendir. Aspir, ayçiçeği, kolza, kenevir ve bakla ise tercih edilmeyen tohumlardandır.

Anahtar Kelimeler: Hasatçı karıncalar; Mevsimsel aktivite; Tohum tercihi; Besin kaynağı; Abiyotik faktörler.

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1. INTRODUCTION

There are 168 valid taxa (134 species, 37 subspecies and 1 fossil species) belonging to the genus *Messor* of harvester ants, and they are distributed in the Palearctic, Afrotropical and Oriental regions (Bolton, 2023). The food source of the ant species grouped as harvester ants is the seeds they collect, so they play a significant role in seed dispersal (De Almeida et al., 2020), particularly in the open, arid and semi-arid areas that they are mainly distributed.

The spread of seeds in nature by harvester ants is called Myrmecochory. There is usually an elaiosome region on plant seeds that looks like a cover or sheath. The elaiosome is an oil-containing region that emits an odour or taste related to the content of the seed. Ants choose seeds by perceiving the nutrient content with the odour or taste they perceive from these regions. Myrmecophorous ants tear off the elaizoma, a soft, oily substance on the seed, and throw away the rest of the seed, making them successful seed dispersers. Although both harvester ants and Myrmecophorous ants have similar seed dispersal roles, the seed regions they feed on are different.

Despite having a wide range of choices in their diet, harvester ants discriminate among seeds to some extent. All other things being equal, there is a tendency to choose the plant with the most abundant seeds (Hahn & Maschwitz, 1985). In a study on the food selection and size of foraging workers of the harvester ant species *Messor bouvieri* (Willott et al., 2000; Silvestre et al., 2019), it was observed that the interaction between the ant and the seed depends on the availability of the seed and the size of the workers.

Ants generally continue to search for food throughout the year. Workers search for food especially in the mornings and at dusk, and are less active during the most humid and hot hours of the day. On the other hand, they are less active at low temperatures. In the summer months, they find food more often at night. This situation can continue in September and October. Ants have the ability to tolerate different temperatures during the day. Since ants are cold-blooded (ectothermic) creatures, temperature changes are important in their activities. For example, species with a high tolerance for heat generally show a diurnal lifestyle, whereas species with a low tolerance limit to heat exhibit a crepuscular - nocturnal lifestyle. Forests and wooded areas exhibit a heterogeneous structure throughout the day in terms of sunbathing and shade. Humid and open areas (meadows, areas with no trees or sparse trees) are more homogeneous and species that tolerate heat are more common in these areas.

According to functional activity changes, daily activity rhythms (circadian rhythm) can increase or decrease in various animals, plants and microorganisms to different degrees and at different times (Elwood & Mcluskey, 1963). Circadian rhythm, which is widely seen in living things, is an example of a biological clock mechanism. It is the regular occurrence of certain physiological events at 24-hour intervals even in the absence of an environmental stimulus such as sunlight. Communities are programmed to comply with

biological rhythms (Elwood & Mcluskey, 1963). Ant activity rhythms are generally adjusted according to environmental factors. Among the abiotic factors affecting activation; temperature (Sanders, 1972), humidity (Sharma et al., 2004), atmospheric pressure, general condition of the air, soil profile, soil moisture, and among the biotic factors, the most important one is vegetation (Sanders, 1972; Sudd & Franks, 1987; Sharma et al., 2004). Seasonal activation rhythms of ants are observed in three stages (Sanches et al., 2005): (1) Preparation for activation (pre – activation) (spring: February to April); (2) Activation (Summer season: April to September); and (3) Diapause (Autumn – Winter: October to February). In this study, we aimed to examine seasonal effects of abiotic factors and nutrient sources on the feeding activity of the harvester ants.

2. MATERIAL AND METHOD

Messor semirufus ants (**Figure 1**) build their nests in a wide variety of habitats such as agricultural lands, meadows, pastures, willow groves, poplar groves, and mixed forests. The nest of *M. semirufus* is a multi-entrance nest with an opening to the outside (**Figure 2**).

Figure 1: *Messor semirufus* workers (Photo: Kaya and Camlitepe, Edirne).

Figure 2: Multiple entry of *Messor semirufus* nest (Photo: Kaya and Camlitepe, Edirne).

Within the Balkan Campus of Trakya University (41.6373486, 26.6112102), activation rhythms and feeding regimes of a *M. semirufus* colony were observed in spring, summer and autumn throughout the year. Abiotic factors (ground temperature, air temperature, air humidity, general weather conditions) were measured every month and the effects of biotic factors such as vegetation were examined and the recorded values were entered into follow-up tables. The plant seeds *M. semirufus* workers preferred from the plants naturally growing in the observed nest habitat were recorded and identified. In addition, 12 different food seeds not naturally found in the *M. semirufus* nest habitat were placed near the nest and seed preferences among these were observed and recorded.

2.1. Monitoring of M. semirufus activation rhythm

The daily activation rhythms of the studied *M. semirufus* colony were recorded for 5 days a week (Monday - Tuesday - Wednesday - Thursday - Friday). Observations and measurements were made at the nest every 2 hours (07.00-09.00-11.00-13.00-15.00-17.00-19.00-21.00). Measurements and observations were made for 9 months in 3 seasons, namely spring: March, April, May; summer: June, July, August; and autumn: September, October, November, and the values were recorded in the relevant monitoring tables. The number of workers in activity was counted around the nest and at the nest entrance, within the area where *M. semirufus* dispersed during food collection. *M. semirufus* workers carrying food were also examined. How often *M. semirufus* collected food, how far from the nest they found the food, and how many worker ants brought the food to the nest were noted within the determined hours.

2.2. Seed preferences of M. semirufus

2.2.1. Order of preference of plant seeds collected from natural environment by M. semirufus

The first exits of *M. semirufus* workers from the nest were for the purpose of reconnaissance tour. First, it was observed which seed they preferred. The priorities of *M. semirufus* workers in seed preference and which seed they preferred more were recorded and compared. A ranking was made according to the order of priority in collecting and carrying seeds by workers to the nest. Seed varieties that *M. semirufus* workers did not prefer were also recorded. The number of paths created by workers during seed transportation was determined. The plants that *M. semirufus* workers preferred naturally in its habitat were observed by following the path columns they created. The plants that these seeds belonged to were identified.

2.2.2. Order of preference of offered seeds by M. semirufus

In the experiments conducted for the preference of different food seeds that are not naturally found in the nest habitat of *M. semirufus*. Wheat, flax, oat, safflower, chickpea, broad bean, sesame, millet, spinach seed, rapeseed, hemp, sunflower seeds were used. These seeds were placed around the determined nest, 1 m away from the nest entrance.

3. RESULTS AND DISCUSSION

The observed *M. semirufus* workers actively started seed collection behaviour in April when the air temperature exceeded 17°C. In the spring, food collection behaviour started around 10:00 and continued until 18:00 in the evening. In July, when the summer temperatures were very effective, it was observed that approximately 1000 worker ants started searching for food and started collecting food at 08:00 in the morning. It was observed that they retreated to the nest at 11:00 when the temperature of the day increased significantly and did not come out until the coolness of the evening. This situation shows that *M. semirufus* workers avoid extreme heat even though it is active in the hot seasons of the year. In autumn, the food collection behaviour of *M. semirufus* workers, which routinely starts around 09:00, continues until 19:00 in the evening. This activation rhythm is consistent with other studies on harvester ants (Elwood & McClusley, 1963; Hölldobler, 1976; Hahn & Maschwitz, 1985; Retena & Cerda, 2000).

3.1. Differences in individual numbers and activation averages of M. semirufus according to seasons

Messor semirufus workers is not active on the soil during the winter months. The average number of workers in the spring season is around 90 (**Figure 3**), the average number of workers in the summer season is around 1200 (**Figure 4**) and the average number of workers in the autumn season is around 350 (**Figure 5**).

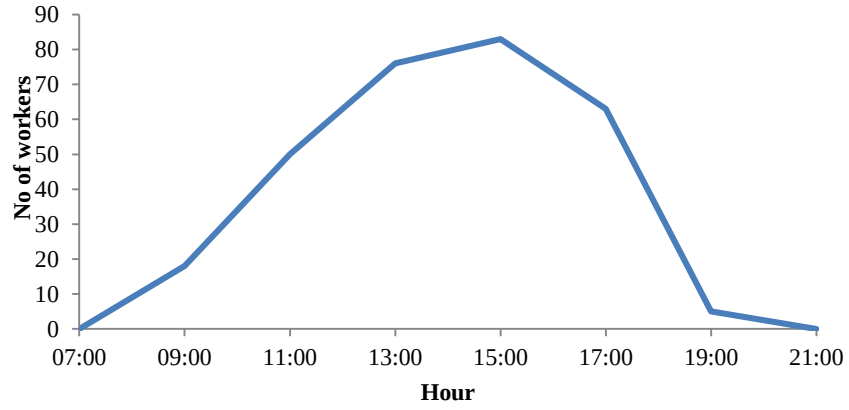

Figure 3: Average number of *M. semirufus* workers active outside the nest in spring.

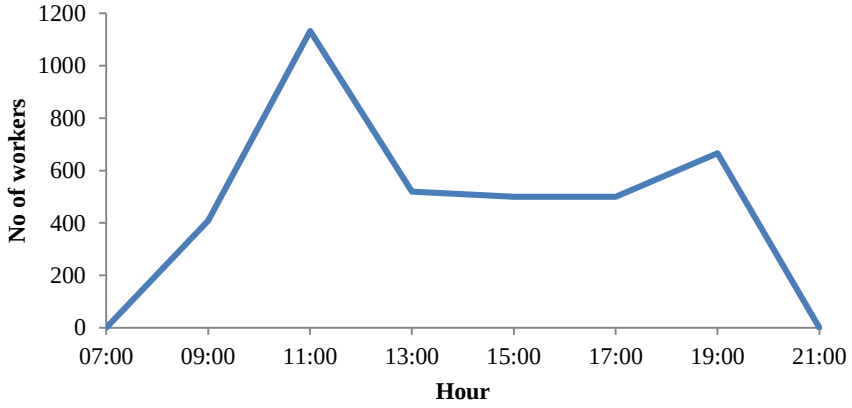

Figure 4: Average number of *M. semirufus* workers active outside the nest in summer.

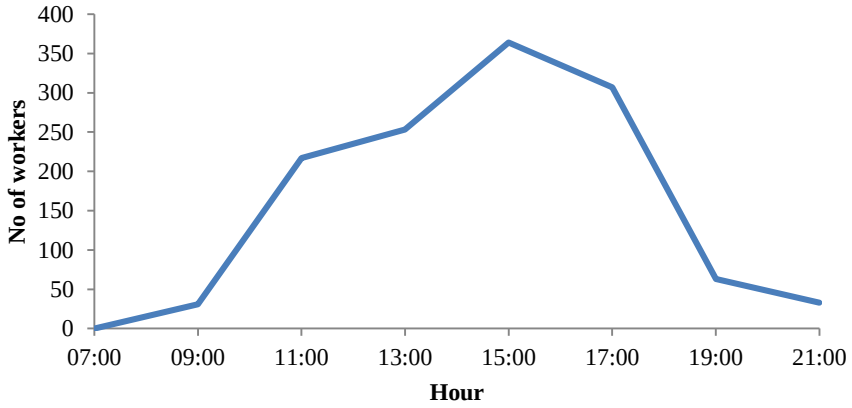

Figure 5: Average number of *M. semirufus* workers active outside the nest in autumn.

3.2. Differences in air humidity rate according to seasons

In the seasons when *M. semirufus* workers are active (spring, summer, autumn), the humidity rate in the air was 47.5% in spring (**Figure 6**), 45-47% in summer (**Figure 7**), and 48-52% in autumn (**Figure 8**). *M. semirufus* workers showed activity at these humidity values, which are close to the average.

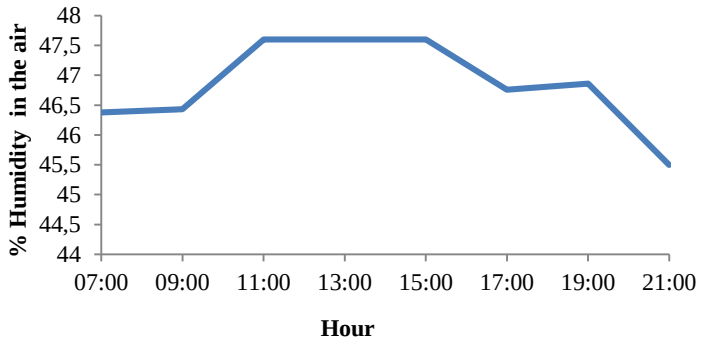

Figure 6: Average humidity in the air in spring.

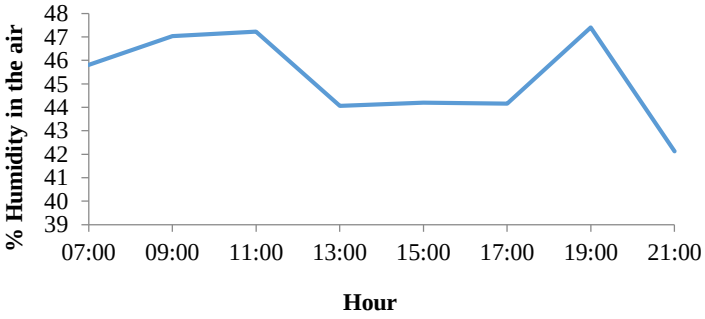

Figure 7: Average humidity in the air in summer.

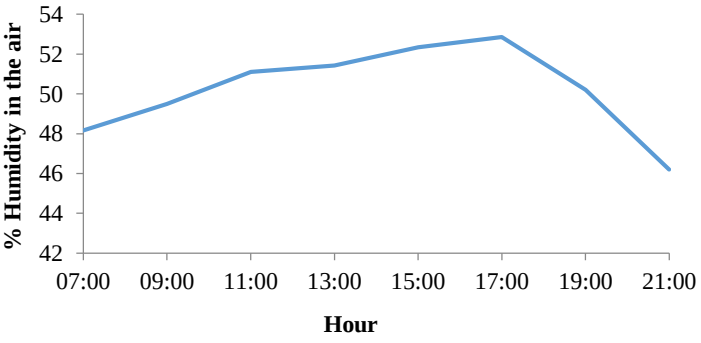

Figure 8: Average humidity in the air in autumn.

3.3. Differences in air temperature according to seasons

The average air temperatures in the seasons when *M. semirufus* workers are active are as follows: 15-20°C in spring (**Figure 9**), 35-40°C in summer (**Figure 10**), and 20°C in autumn (**Figure 11**). The number of workers counted was also highest in the summer season when the temperature was highest.

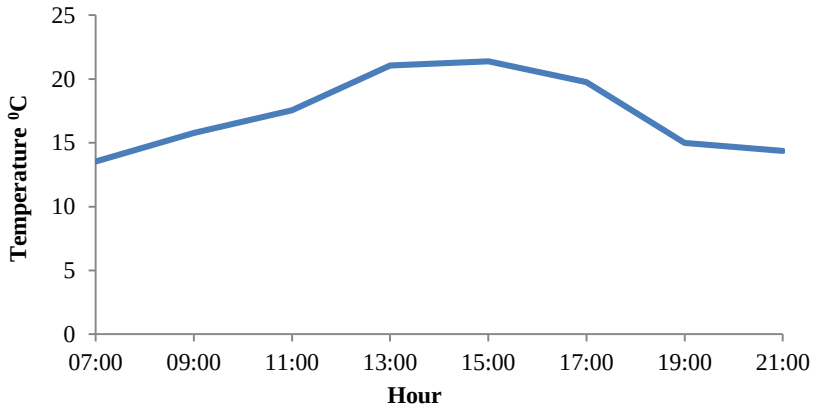

Figure 9: Average air temperature in spring.

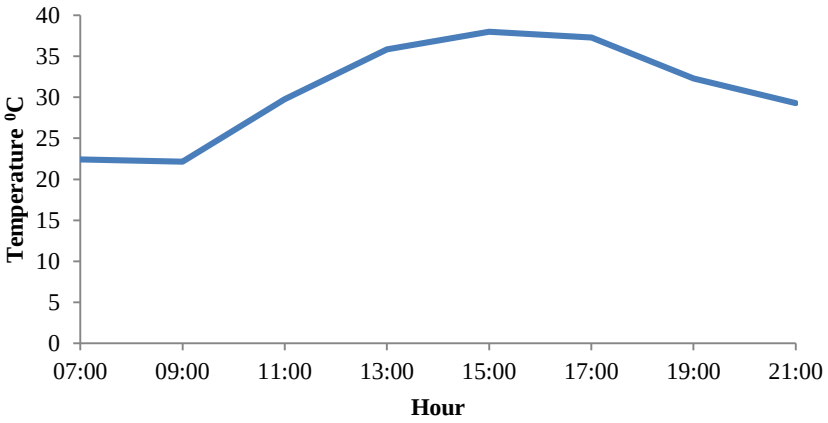

Figure 10: Average air temperature in summer.

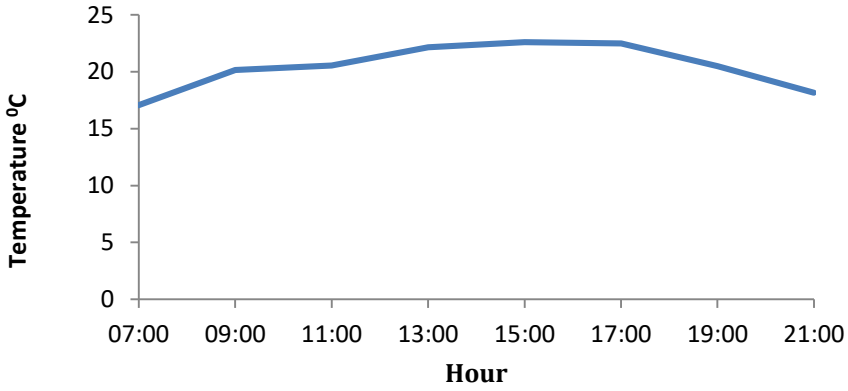

Figure 11: Average air temperature in autumn.

3.4. Soil temperature differences according to seasons

Soil warming is one of the important abiotic factors for ants. *Messor semirufus* workers examined are not active in conditions where the soil temperature is below 4°C. The average soil temperatures in the seasons when *M. semirufus* workers are active are as follows: between 12-14°C in spring (**Figure 12**), over 40°C in summer (**Figure 13**), and between 16-18°C in autumn (**Figure 14**). The summer season, when the soil temperature is above 40°C, is the period when *M. semirufus* workers are most active.

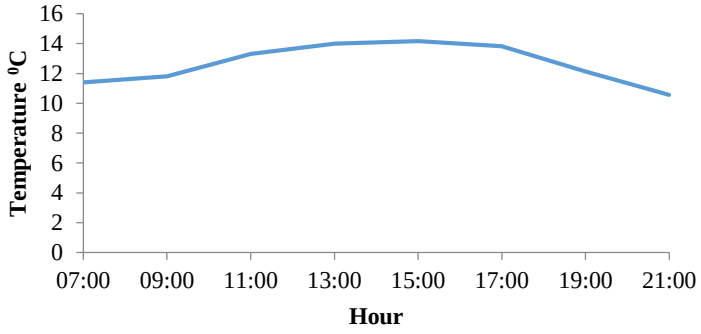

Figure 12: Average soil temperature in spring.

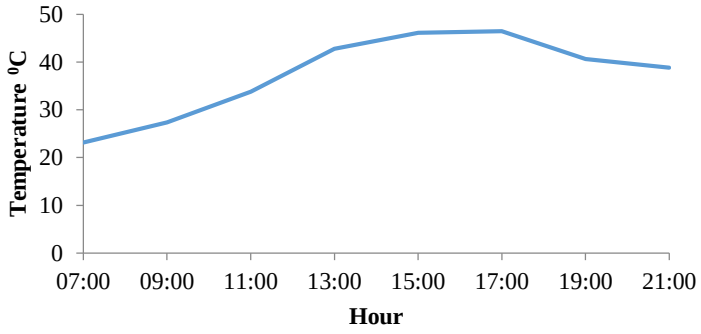

Figure 13: Average soil temperature in summer.

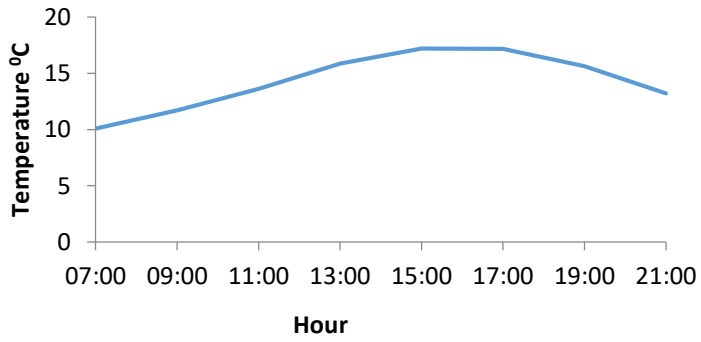

Figure 14: Average soil temperature in Autumn

3.5. Preference of M. semirufus workers for plant seeds collected from the natural environment

Five species from the Poaceae (grass) family were determined to be preferred by *M. semirufus* and these species are: *Seteria glauca, Cynodon dactylon, Cynadon dactylon villosus, Sorghum halepense* and *Poa trivialis. M. semirufus* workers also carried seeds from the Asteraceae family, which is the second most common in the environment. In the observed environment, the species belonging to the Asteraceae family were *Lactuca serriola, Conyza canadensis, Senecio aquaticus and Helminthotheca echioides*. Apart from the Asteraceae and Poaceae families, other plant species preferred by workers are *Polygonum aviculare (Polygonaceae), Anagallis arvensis (Primulaceae), Oxalis corniculata (Oxalidaceae), Euphorbia chamaesyce (Euphorbiaceae)* and *Portulacea oleracea (Portulaceae).* During the observations, it was determined that the trails created by *M. semirufus* workers were divided into 4 branches at different distances. These trails were of different lengths: 3 m, 4 m, 6 m and 8.5 m. This shows that if there is more than one food in the habitat, *M. semirufus* tends to notice and collect all of them at the same time.

3.6. Offered seeds preferences of M. semirufus

On the days when *M. semirufus* was active, 12 different seed options were placed in equal amounts (each 200 gr.) 1 m away from the entrance of the observed nest. The plant seeds left were as follows: wheat, flax, oat, hemp, chickpea, broad bean, rapeseed, sesame, sunflower, millet, safflower and spinach seeds. In July, at around 07:00 in the morning, two minor workers went out to scan the area. They took one flax seed each and returned to the nest. It took them about half an hour to scan the area and return to their nests. At around 07:30, an average of 200 *M. semirufus* workers had tended to each seed. About half an hour later, at 08:00, 1000-1500 *M. semirufus* workers were mobilized to carry the seeds to the nest.

Among the *M. semirufus* colony members that collect seeds, minor workers predominate. Of the workers that create trails while collecting seeds, on average one in every 30 is a major worker. In an environment where an average of 1500 workers are actively collecting food, there are 50 major workers, while the others are minor workers. Major workers collected larger seeds such as oat, wheat, safflower, and spinach, which were larger than the others out of the 12 seeds presented (**Table 1**). Minor workers are quite rare among the workers that collect large seeds. The transportation of flaxseed, which is their primary preference, was done only by minor workers (**Table 2**). Workers who looked at all seeds during the first round of exploration soon abandoned sunflower seeds, hemp, broad beans, safflower and rapeseed and turned to other seeds.

The order of preference of the seeds offered by *Messor semirufus* is as follows (see **Table 1**):

(1) Wheat: Among the twelve seeds, their first priority was wheat seed. They finished carrying the 200 grams of seeds to the nest within 45 minutes. It can be said that wheat seed is their first choice because it has the shortest seed transportation time. Even if they continued to carry other seeds, they focused on carrying wheat first.

(2) Flaxseed: Their second choice was flax. Seeds with high oil content: Flax, sesame, sunflower and safflower. Flaxseed is the favourite among seeds with high oil content. It took about 60 minutes to transport flaxseed.

(3) Oat: It is the third most carried oat seed. It took them about 90 minutes to carry the oat seed to the nest.
(4) Spinach Seed: It is the seed they carry to their nest in the fourth place. The carrying activity continues after the wheat, flax and oats in the environment are finished. They were mostly carried by major workers. Carrying the spinach seed continued throughout *M. semirufus*'s one-day activities (**Table 1**).

(5) Sesame: This is the seed that can be ranked fifth. *It was M. semirufus'* second choice among seeds with high oil content. They collected half of the 200 g of sesame seeds offered within two days.

(6) Chickpea, Millet: They showed very little interest in chickpea and millet seeds. They rarely visited these seeds for two days. The number of seeds they collected from both seeds for two days was around 9-10. When millet seeds were first left, they tended to move more than chickpea seeds, but they abandoned millet seeds because they had difficulty carrying them. As a result, the number of seeds taken to the nest from these two seeds is very close to each other.

(7) Safflower, Sunflower: These are the seeds that workers showed very little interest. On the first day they were left, workers took 3-4 seeds from both seeds to the nest. No carrying behaviour was observed from these seeds on the other days.

(8) Rapeseed, Hemp, Broad Bean: There is no interest in these three seeds. Workers never carried them to the nest. Except for 5-6 workers who walked over them during the first exploration, they never visited these seeds again. Hemp, rapeseed and broad bean seeds were recorded as non-preferred seeds (**Table 1**).

When *M. semirufus* workers encountered the first seeds, they went back and forth between the nest and the food in a mixed manner. Within half an hour, the tendency towards their first three choices, wheat, flaxseed and oats, increased significantly and three paths were clearly formed. It was observed that very few workers went back and forth to the seeds that came after these three seeds, without any obvious path.

Table 1: Order of preference of offered seeds by *M. semirufus* workers.

Order of preference	Seeds name	Amount of seed dropped	Number of seeds collected	Time for collecting seeds (minutes-days)
1	Wheat	200 gr	All collected	45 minutes
2	Flaxseed	200 gr	All collected	60 minutes
3	Oat	200 gr	All collected .	90 minutes
4	Spinach	200 gr	All collected .	1 day
5	Sesame	200 gr	Half of it collected	2 days
6	Chickpea, Millet	200 gr (each)	9-10 pieces were collected	2 days
7	Safflower, Sunflower	200 gr (each)	3-4 pieces were collected	1 day
8	Rape, Hemp, Broad bean	200 gr (each)	No seeds were collected	-

Table 2: Seeds collected by worker size of *M. semirufus*.

***M. semirufus* worker size**	**Seed names**
Seeds Collected by Major Workers	Oats, Spinach, Safflower, Wheat, Sunflower
Seeds Collected by Minor Workers	Wheat, Flaxseed, Chickpea, Millet, Sesame, Oat

4. CONCLUSION

The *M. semirufus* colony, which was monitored throughout the year, was determined to be active on the soil in spring, summer and autumn. In the observations, it was observed that *M. semirufus* spent the winter season and the first month of spring, namely December, January, February and March, underground. The observed *M. semirufus* workers chose a safe habitat to protect themselves from the extreme summer heat by building their nests in a secluded corner. Again, in the observations, the ants preferred sheltered places to protect themselves from abiotic factors, but it is thought that the main factor for building nests is food. The reason why the *Messor* genus is common in regions with grain fields in agricultural lands is the accessibility of food sources. In the observation made on the days when the average temperature in March was 15°C, it was observed that *Formica cunicularia*, another ant species which is neighbouring the *M. semirufus* nest, came out of the nest and showed activity on the soil (foraging, nest repair), while *M. semirufus* (generally valid for the *Messor* genus) workers had not yet come out of the nest and were not active in any way. This situation shows that the *Messor* genus is a more heat-loving species compared to the *Formica* genus. In fact, this situation confirms the statement that harvester ants are desert harvesters, which is expressed in many countries (De Vita, 1979; Brown et al., 1979a, b; Davidson et al., 1984).

In the year when the observations were initiated, the average temperature of March, which is the first month we started, was around 15°C. The observed *M. semirufus* workers started actively collecting seeds in April when the air temperature exceeded 17°C (see **Figure 9**). The food collecting behaviour started around 10:00 in the spring and continued until 18:00 in the evening (see **Figure 3**). In July, when the summer temperatures were very effective, it was observed that approximately 1000 worker ants went out to search for food and started collecting food at 08:00 in the morning (see **Figure 4**). It was observed that they retreated to the nest at 11:00 when the temperature of the day increased significantly and did not come out until the coolness of the evening. In summer, food searching and food carrying activity was observed for about one or two hours as of 19:00 in the evening. This shows that *M. semirufus* avoids extreme temperatures even though it is active in hot seasons throughout the year. In fact, it has been observed that on days when the air temperature exceeds 40°C, there is a period of about a week when they do not leave the nest during the day except for food gathering activities between 08:00 and 11:00 in the morning. In autumn, the food gathering behaviour of *M. semirufus* workers starts routinely around 09:00 and continues until around 19:00 in the evening. The time period when the most of the individuals are counted during this routine activation is between 11:00 and 17:00 (see **Figure 5**). In this study, it was observed that the activation of *M. semirufus* during the day varies according to the seasons. Our results support the study of Hunt (1974) who reported that there are differences in activation in ants during the day and depending on the seasons. In this work, it has been observed that *M. semirufus* workers tend to collect all seeds, whether they are in their natural habitat or experimentally added to the environment. During the seed collection process, *M. semirufus* workers are selective in their seed preferences. It has been observed that they tend to collect seeds that are present in their environment during the period when they are generally active. The reason why they collect seeds of plants in the Poaceae family more in spring and summer is that they can find the plant more frequently in these periods. Based on our observations, the fact that the most seeds from the Polygonaceae, Primulaceae, Oxalidaceae, Euphorbiaceae, Portulaceae and Compositeae families are collected by *M. semirufus* workers in summer and autumn is due to the availability of the seeds of the plants in these seasons. Seed production by Asteraceae members in three seasons has ensured that they are preferred by *M. semirufus* workers throughout the three seasons.

The experiment in which seeds were left near the *M. semirufus* nest and seed selection was observed was carried out in spring, summer and autumn and workers collected the seeds they found ready in all three seasons. For instance, they preferred flaxseed, which is their primary preference, in summer and in the same way in autumn. It was revealed that rapeseed, hemp and broad bean seeds, which are not preferred seeds, were not preferred by the ants when the environment was low in nutrients. It was observed that workers carried the seeds they preferred as food to the nest regardless of the season (Nondillo et al., 2014).

The distances between the food found by the ants collecting seeds and the nest may be different. While some of the food they find is as close as 1 m to the nest mouth, some may be 10 meters away. It was observed that *M. semirufus* workers who set out to explore in search of food noticed the food near the nest entrance? within 10 minutes and took one seed and carried it to the nest. Later, it was determined that the other worker ants in the nest followed each other and headed towards the food and created a path between the nest and the food. During the study, the food that was farthest was 10 m away and it took them about half an hour to notice this food during the exploration tour. *Messor semirufus* workers can head towards more than one food at the same time and create more than one path tract (Hölldobler & Möglich, 1980). During the observations, it was observed that the number of paths formed according to the food availability changed; sometimes there was only one path, sometimes 2 or more paths were observed. It was also witnessed that the path traces formed by *M. semirufus* workers divided into up to 4 branches at different distances. These paths have different lengths: 3 m, 4 m, 6 m and 8.5 m. This shows that if there is more than one food in the habitat, *M. semirufus* tends to notice and collect all of them at the same time.

Among the 12 seeds offered to the *M. semirufus* colony, the workers' first choice was wheat seed. Workers, who also continued to carry other seeds, primarily carried wheat completely. This shows that *M. semirufus* prefers wheat as a food. Wheat seeds were an important food for *M. semirifus* throughout the study. Wheat has a carbohydrate value exceeding 65% in terms of nutritional content (Zeleny et al., 1971). In the context of the study, it was thought that the fact that wheat is a food with a high carbohydrate content provides an explanation for the priority preference of this seed by the ants. In addition, the food with the highest carbohydrate content in the environment was wheat seed. In fact, it was observed that ants preferred high-carbohydrate food to low-carbohydrate food (Nondillo et al., 2014). It was observed that *M. semirufus* workers turned to other seeds after the wheat seed was depleted. After the wheat seed was depleted in the environment, wheat was added to the environment again as soon as *M. semirufus* workers collected the other seeds. The addition of wheat was immediately noticed by the ants (within 1 minute) and the wheat seeds started to be carried from the environment again. This situation reinforces our idea that wheat is the primary food for *M. semirufus*. During this time, *M. semirufus* workers did not completely stop collecting other seeds other than wheat (especially flaxseed). When the wheat seed, which is the first choice of *M. semirufus*, was mixed with the rapeseed seeds that they had never collected and placed around the nest, it was observed that they preferred wheat seed from this pile and did not prefer rapeseed. Of these mixed seeds, rapeseed was the only one left. When a mixture of wheat, hemp and rapeseed was given, it was observed that the ants took the wheat and did not take the rapeseed and hemp from the environment, which shows that these ants can distinguish the food they use even if they are mixed. When all 12 seeds were given mixed, the ants preferred wheat seeds as usual.

The second choice of *M. semirufus* was flaxseed. Among the seeds with high oil content, flaxseed is the most preferred. In the observations, flaxseed, which is lighter than other seeds, has generally been preferred by minor workers in our study (see **Table 2**) which is consistent with Willott's work (Willott et al., 2000). In addition, spinach plant, which is frequently grown in Turkey, was chosen to observe whether these ants prefer the seeds of green vegetables. *Messor. semirufus* workers continued to carry spinach seeds after the wheat, flax and oat seeds in the environment were depleted, showing that the ants give more importance to carrying seeds, which is their first priority. Spinach seeds have not generally received much attention like wheat, but they are still among the seeds carried to the nest. This situation shows that they tend to collect and store other foods even if they are a food they like more in the environment. In the study of Aktaç et al. (1990), the seed stores of *M. semirufus* were investigated and plant seeds belonging to 6 plant families were found, proving that they want to store a wide variety of seeds. Spinach seeds were mostly carried by major workers because they were the larger seeds than other seeds in the environment. During the study, it was observed that large seeds were carried by major workers. In fact, similar results were reported by Willott et al. (2000) in harvester ants of the species *Messor bouvieri*.

When millet seeds were first dropped into the environment, they were a type of seed that had a high tendency to move but could not be carried to the nest very much. When this seed was first dropped, it was tried to be carried and dropped a lot. This situation occurred because the millet seed was round and smooth. Ants could carry very few seeds from among the millet seeds. They carried about 10 seeds during a day.

The grinding of chickpeas caused the elaisomal region to break down. The elaisomal part of the broad bean is in the shell on the grain. The peeling and grinding of the dried broad bean prevented it from being noticed by the ants. It is thought that the dispersion of the elaisomal region, where the ants perceive the content of the food, may have prevented *M. semirufus* workers from preferring chickpea and broad bean seeds. Safflower and sunflower seeds are also among the seeds that are preferred very little. Only 3-4 of them were carried in the environment and were not carried again. The fact that safflower and sunflower were shelled prevented them from being preferred.

Messor semirufus workers showed no interest in rapeseed and hemp seeds and never carried them to the nest. After these seeds were left, workers walked over them when they first noticed them. They took a few rapeseeds and left them back and never carried them to the nest. The oil of the rapeseed product contains erucic acid and glucosinolate, which are harmful to human health, and this seed is not preferred by the ants. Erucic acid above the specified value causes fattening of the heart muscles, and high levels of glucosinolate in the pulp of the rapeseed plant cause thyroid gland enlargement, gout inflammation and liver disorders in animals. Glucosinolates are water-soluble, anionic, non-volatile and heat-stable compounds (Artik & Yemiş, 2006). When plant tissue is broken or damaged by various processes (such as cutting, chopping or chewing), glucosinolates are hydrolysed by endogenous myrosinase naturally present in the tissue, and as

a result of this degradation, degradation products such as isothiocyanates, thiocyanates, nitriles, oxazolidine-2-thiones, hydroxynitriles, and epithionitriles are formed (Artik & Yemiş, 2006). The reason why hemp seeds are not preferred by *M. semirufus* workers is that the ants perceive the seed content. In a study conducted in Brazil, the preference of *Tenebrio molitor* (mealworm) and *Gryllus* (grasshopper) species, which were given to the ant species *Lasius micans* as food, for the *Gryllus* species, which has a high protein value, indicates that they perceive the nutrient content (Nondillo et al., 2014). If *M. semirufus* harvester ants did not perceive the content of the seeds, they would be expected to collect rapeseed, hemp, and broad bean seeds indiscriminately and take them to their nests. However, *M. semirufus* has been observed to hold rapeseed, hemp and broad bean seeds with their mandibles for a short time and then release them. This supports the view that ants perceive the content of the food when making their choice. Otherwise, they would be expected to collect rapeseed, hemp and broad bean seeds as they collect wheat seeds.

During these observations using seeds, on another day, *M. semirufus* workers were not given their first two choices, wheat and flax seeds, at all. Instead, they were given hemp, rapeseed, spinach, chickpea, broad bean, oat, sunflower, safflower, millet and sesame seeds. The workers carried some of the spinach and oat seeds but did not touch the other seeds. When their first two choices were not in the environment, they collected their third and fourth choices and did not carry the seeds that they had previously perceived to be.

The species whose seeds were preferred by *M. semirufus* from the Poaceae (wheat) family are: *Seteria glauca, Cynodon dactylon, Cynadon dactylon villosus, Sorghum halepense, Poa trivialis.* There were no plants from the Poaceae family other than these five species around the observed *M. semirufus* nest. *M. semirufus* workers collected seeds from plants belonging to the Poaceae family throughout the spring season. Seed collection behavior continued from the drier Poaceae family in the summer season.

M. semirufus workers also carried seeds from members of the Asteraceae family, which is the second most abundant in the environment. *Lactuca serriola, Conyza canadensis* and *Helminthotheca echioides* were naturally grown in the observed environment as Asteraceae members, and the ants carried seeds from all three species. The seeds of the Asteraceae family are in a volatile form and are pinnate and they are collected by *M. semirufus* workers in all three seasons (spring, summer, autumn), except winter. The Asteraceae and Poaceae families are the most abundant and preferred plants in the observed habitat.

Other preferred plants besides Asteraceae and Poaceae families are: *Polygonum aviculare* (Polygonaceae), *Anagallis arvensis* (Primulaceae), *Oxalis corniculata* (Oxalidaceae), *Euphorbia chamaesyce* (Euphorbiaceae), *Portulacea oleracea* (Portulaceae), *Senecio aquaticus* (Compositeae). These species are generally preferred by *M. semirufus* because they produce seeds in summer and autumn. In a study investigating the nest type and food stores of *M. semirufus* (Aktaç et al., 1990), the main seeds detected in

the food store belong to the families Ranunculaceae, Polygonaceae, Leguminosae, Boraginaceae, Rubiaceae and Graminae. According to our results and this study, the only family that is common among the preferred families is Polygonaceae. The reason why the families Ranunculaceae, Leguminosae, Boraginaceae, Rubiaceae and Graminae could not be detected in the food stores in the study conducted by Aktaç et al. (1990), may be due to the absence of these families in the habitat studied. There are also plants that *M. semirufus* does not prefer even though they are near the nest. These are *Mentha pulegium* (Lemiaceae), *Lotus corniculatus* (Fabaceae), *Trifolium repers* (Fabaceae). The reason why the Fabaceae and Lemiaceae families are not preferred may be that they do not have obvious seeds on the plant.

As a result, our findings revealed that *M. semirufus* is selective in seed preference while collecting seeds in the spring, summer and autumn seasons when it is active *M. semirufus* prefers seeds with high carbohydrate and protein values. *M. semirufus* collects seeds from the families Asteraceae, Poaceae, Polygonaceae, Primulaceae, Oxalidaceae, Euphorbiaceae, Portulaceae and Compositeae. The seeds they collect the most in nature are grasses and the data confirming this finding was obtained in our experimental study. Similar new studies that will reveal the similarities and differences in food preferences of species will undoubtedly make great contributions to the determination of the special strategies developed by species in food competition. These contributions will be of great importance in elucidating the feeding ecology of harvester ants, which play an important role in the ecosystem and play an active role in the distribution of seeds.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest among the authors.

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CONTRIBUTIONS OF AUTHORS

S.E.K: Methodology, software, validation, formal analysis, investigation, resources, writing—original draft preparation.

Y.C.: Conceptualization, Methodology, validation, investigation, resources, writing—review and editing.

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