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Seasonal response of feeding, differentiation, and growth in the eastern subterranean termite *Reticulitermes flavipes* (Kollar) in Wisconsin

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Seasonal response of feeding, differentiation, and growth in the eastern subterranean termite *Reticulitermes flavipes* (Kollar) in Wisconsin

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ABSTRACT
In termites, differentiation plasticity in undifferentiated *Reticulitermes* progresses with growth stages from larvae to workers, which may then differentiate into soldiers, winged nymphs, or neotenics. Although studies have been done on seasonality of the termite life cycle, data appears to vary from location to location. *Reticulitermes* populations in Wisconsin appear to behave slightly differently in terms of colony formation and differentiation than in other parts of the continent. Timing of differentiation, feeding, and foraging behavior show some specific trends in Wisconsin termite colonies, probably linked to climatic limitations. Peak egg laying is seen in April to May, followed by a peak in emergence of larvae in May, with primary growth and differentiation occurring in June and July. Growth, feeding, and differentiation all drop off around late July to early August, reflecting an overwintering population of mainly workers.

Keywords: Caste differentiation, *Reticulitermes flavipes* (Kollar), feeding, seasonal variation

1. INTRODUCTION
Few studies are related to seasonal variation on termite capabilities and changes within a population. Such information on *Reticulitermes* is usually associated with monthly or seasonal observations of natural populations or based on non-calendar, non-repetitive experiments. These observations, while noting general trends, require a more focused study to show a complete pattern of caste differentiation.

Previous work has indicated that termites show an annual fluctuation of composition in caste forms that tend to follow a pattern (Esenther 1969). Formation of various castes among termite families can vary dramatically. For example, it is frequently found in the Rhinotermitidae that new colonies are developed by the process of fission in which substitute reproductives form in a portion of the colony that has been isolated from the main body (Harris 1971). To the same degree, termites belonging to this family in the genus *Reticulitermes* are shown to use this distribution method in preference to swarming (Harris 1971). Therefore, alate forms are encountered less frequently in *Reticulitermes* colonies, and these closely distributed colonies can take up a larger area.

Reticulitermes flavipes (Kollar) develops by incomplete (hemimetabolous) metamorphosis and undergoes a caste differentiation process that is unlike other social insects. Workers in this genus have three developmental potentials: regressive molts, in which they develop into worker-like pseudergates, imaginal (winged) alates, or apterous (wingless) reproductives that supplement the reproductive capacity of the colony (Scharf *et al.* 2003). Workers also have three developmental potentials: molts that retain them as workers, differentiation into the white soldier stage immediately followed by the soldier stage, or differentiation into apterous third-form reproductives, which also function to supplement the reproductive capacity of the colony (Scharf *et al.* 2003).

Caste polymorphism and plasticity is unique in termites. Termites in the *Reticulitermes* are considered lower termites on the basis of two characteristics: one being their dependence on protozoan symbionts for digestion of cellulose, and the second involving the status of the worker caste being a highly unfixed, immature stage (Zhou *et al.* 2006). This variability in colony formation has resulted in increased interest in the factors influencing caste differentiation and has been a main focus of many termite studies.

The objective of this study is not to analyze the mechanisms influencing caste formation, but rather to provide observations on the variable annual changes in the caste forms within an active population in Wisconsin and the potential of samples from the population in terms of growth, differentiation, and weight loss of a food substrate. It may then be possible to apply this information when considering baiting treatment of termites in the state, as these treatment methods are based on knowledge of basic termite biology and behavior. It may also provide insight on how to improve in-lab termite testing and accuracy of results.

2. MATERIALS AND METHODS

The termites for this study were from the super colony population of *R. flavipes* at Janesville, Wisconsin, in 1979 and 1980 (A super colony is a population with multiple reproductive sites with an interconnecting tunnel system.). Corrugated cardboard traps were used to collect termites weekly at multiple (usually seven) sites in the first season and up to fourteen sites in the second season (Fig. 1). Experimental methods involved two basic set ups, one-gram samples of collected termites, and 50 worker groups selected from these trapped termites. Both of these techniques are described further below.

Figure 1: Corrugated cardboard trap

2.1 One-Gram Samples

For each of the weekly collection sites in season two, the gross weight of the termites was averaged, and a one-gram sample was selected at random from the collected termites to provide an estimate of the number of termites collected and the average size of individuals and number of termite forms/differentiators in the samples. The average number and composition of differentiators were calculated to establish a basic understanding of the make-up of the colony in the field at various times in the recorded season.

2.2 50-Worker Groups

Depending on collectability, groups of 50 workers were placed in plastic containers along with a water-moistened food source. In season one, initially five replicates (later three replicates) from each of (up to) seven collection sites were placed in the containers from 14 weekly collections (June to November) for a total of 470 units. In season two, usually three replicates from four collection sites were set in containers from 29 weekly collections (April through October) for a total of 362 units.

Each of the 50-worker units had an initial group weight to determine the average size of the workers. Thereafter, each unit was dismantled weekly for 12 weeks to obtain the number and group weight of survivors, the total numbers for any differentiating, and food (oven-dry) weight losses. Any eggs found were removed during these inspections. The means for this data are given for 0- to 4-, 5- to 8-, and 9- to 12-week periods. Only the total number of differentiators during 0 to 12 weeks are provided. Definitions of differentiation terms as used in this paper are given in Table 1.

Table 1: Differential forms of a termite colony (Esenther 1969)

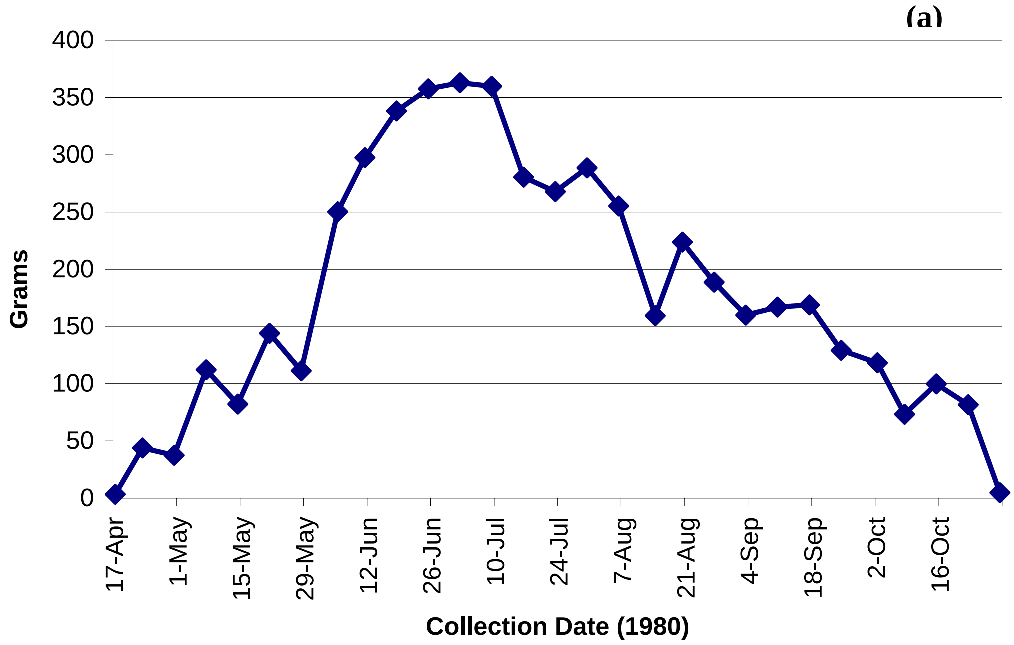
Term	**Description**
Larva	An undifferentiated 1st to 3rd stage nymph
Worker	An undifferentiated nymph in at least the 4th instar
Soldier	A callow or normally pigmented soldier form (Most of the white soldiers in this study represent the presoldier stadium of Weesner (1956) rather than a callow soldier.)
Nymph	A nymph with developing wings projecting as buds from the posterior of the 2nd and 3rd thoracic segment
Neotenic	An apterous or brachypterous reproductive

3. RESULTS

3.1 One-Gram Samples

The total number of grams as well as total number of individuals collected at the specific collection dates are presented in Fig. 2 (a and b).

Data of total number of grams collected in the field in the second collecting season show a clear increase in activity from late May to mid July. This could be indicative of increased foraging to obtain food for the peak of larvae that appear during that same time (Fig. 3). The graph illustrating the number of individuals in one gram agrees with this concept because more termites make up the set from April to May. As foraging and subsequent feeding increase, the larvae and other members of the colony gain weight, so less individuals make one gram. The differentiators seen in these groups express various peaks throughout the season. There is an obvious peak in the number of larvae produced in late April, with a rapid drop in early May. This is the only peak in larvae seen in the collecting season. Although soldier numbers tend to be small, their peaks do show an obvious alternating pattern to those of the nymphs. Overall, soldier differentiation does appear to occur in a fairly stable pattern. These trends reflect changing conditions in a population, which is associated with reproduction, growth, and differentiation or the absence of these factors in the population. The average number of differentiators in these one-gram groups throughout the collection period is presented in Fig. 3.



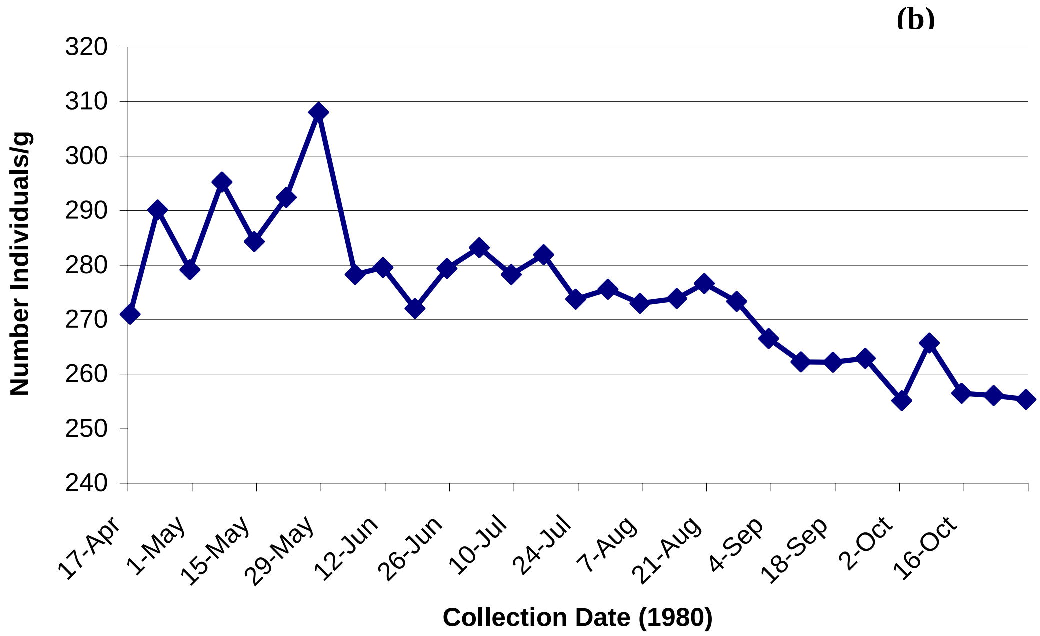


Figure 2: Total number of grams collected (a) and number of individuals in a one-gram sample (b) at various collection dates in 1980

The average size of termites in the one-gram groups at dates of collection were also recorded and were found to vary between 3.5 mg early in the collection period and 4.0 mg per termite later in the season. This variability reflects overwintering populations of workers only; peak of egg laying in early April, progressing to peak hatching in late April to early May, followed by primary growth and differentiation in June and July, and primarily slow growth leading to the overwintering population of probably mainly workers.

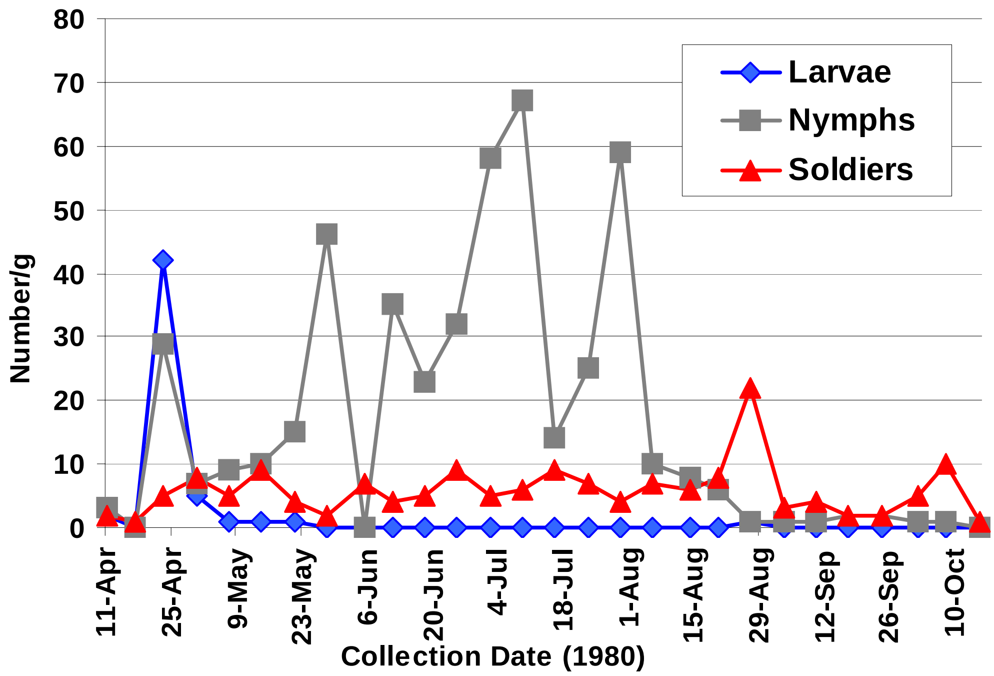


Figure 3: Average number of differentiators in one-gram samples at collection dates in the second collecting season (1980)

3.2 50-Worker Groups

The undifferentiated functional workers showed development into the differentiating forms in the 50-worker units. The nymphs had some wing development, appearing mostly in the 0- to 4-week period. The soldiers have a pre-soldier (i.e., white soldier) stage before the soldier stage that appeared mostly in the 5- to 8-week period. The neotenic males and females, which are the sexually functional forms, developed mostly in the 9- to 12-week period. Differences in differentiation in the three time periods coincide with the number of molts necessary to reach a certain caste form. The differentiating forms, however, were especially prone to cannibalism, thus an unknown number may have formed and been eaten before they could be counted. Cannibalism may account for the variable peaks seen in nymphs and soldiers in the one-gram collection samples, and could represent a significant factor in population nutrition.

Data illustrating percentage survival by numbers show averages of 96.5% up to four weeks, 89.3 % up to eight weeks, and 76.3% up to twelve weeks. Percentage weight per termite of survivors, however, shows a slight increase in weight over the 12-week period. This agrees with the data from the one-gram groups analyzing the number of individuals making up a single gram.

The percentage survival in terms of group weight tends to be slightly higher than percent survival by numbers, reflecting the growth of termites during the 0 to 12-week periods. The greatest difference occurs in the April collection groups and tends to taper off through the progressive collection periods. This tapering off may reflect the condition of the workers at the time of collection as affected by the cycle of reproduction, growth, and differentiation, and mostly slower growth in the Janesville population.

These changes through the year are reflected in the variable-food weight-loss data. Each collection date represents one set of termites, and food weight loss was measured for the group at the three time periods. The most change was observed during the 0- to 4-week period, followed by relatively stable variability of the data in the 0- to 12- and 9- to 12-week periods. The 0- to 4-

week data most closely represents feeding of termites in the field, while reduced feeding observed in subsequent time periods may be due to being placed in an artificial, in-lab environment. This data is useful to consider when doing experiments with termites held in an in-lab colony for over 4 weeks. It can therefore be concluded that termite testing should be done with termites that have been recently removed from the founding colony. Results from the food weight loss test are presented in Fig. (4). The peak of the 0- to 4-week weight losses coincides with the peak period of differentiation in the population (Fig. 3).

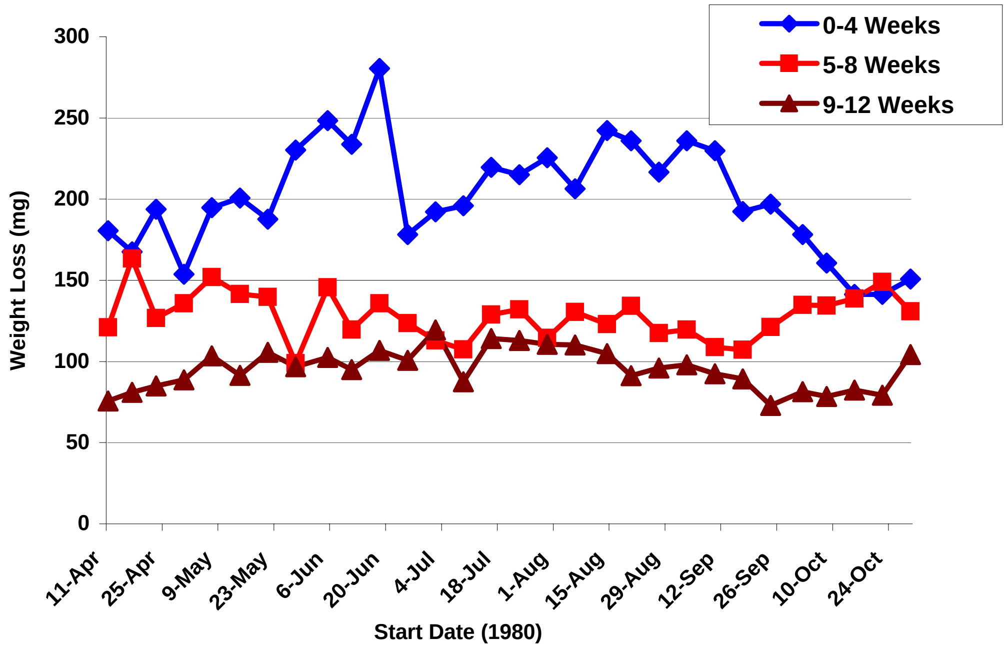


Figure 4: Weight loss of food blocks in second collecting season

Differentiation in the 50-worker groups shows some nymphal development throughout the first season collections, but almost none in the second season collections. This may reflect a population under stress from heavy removal of termites by collections. These collections probably surpassed one million individuals in the first year and an estimated 1.6 million in the second year. (This is based on numbers in a one-gram sample times the total grams collected.). These collections undoubtedly had a significant impact on the ongoing nutritional status of the population.

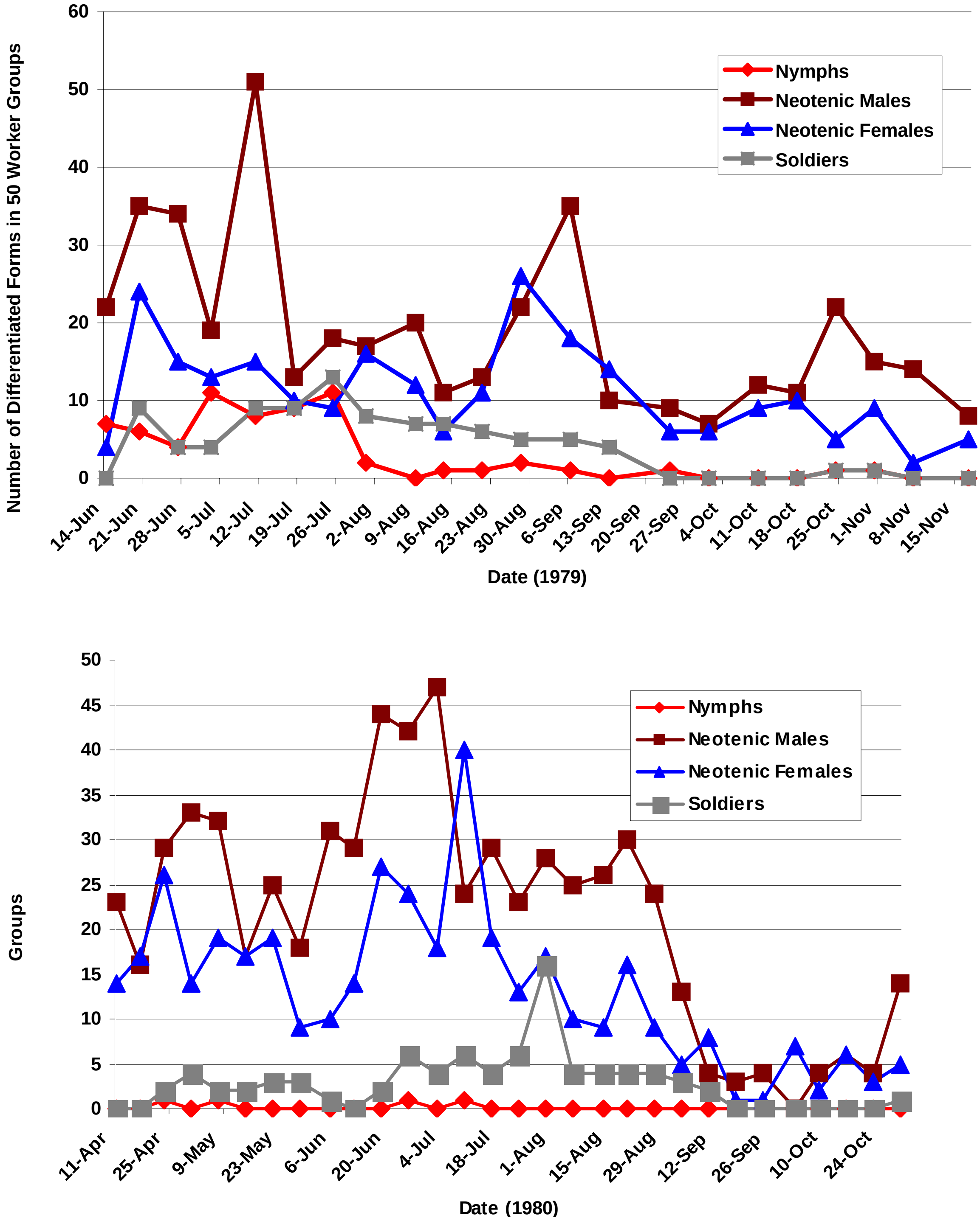


Figure 5: Total numbers of differentiated forms in 50-worker groups in 1979 (a) and 1980 (b) during the 12-week periods

The development of soldiers rarely surpassed 10 individuals in these groups in both years, but as mentioned previously, the peaks of soldier development tended to precede or follow the peaks of nymphal development. The production of neotenic forms was highly variable for both males and females and surpassed considerably the production of nymphs and soldiers. Overall, the differentiation data seems to show that nymphal development is associated with the shortest intermolting period and the soldiers with an intermediate intermolting period. The neotenics are associated with a somewhat longer intermolting period, thus there seems to be a drop off in potential of this type of development as individuals age (Fig. 5 a and b).

4. SUMMARY

Harris (1971) has stated that termites in the genus *Reticulitermes* form new colonies through isolation of part of an existing colony, and subsequent development of substitute reproductives rather than formation of winged adults. Our study agrees with this idea, as we observed an overwhelming production of sexually mature neotenics with no indication of alate production in the field or the laboratory. This type of information is exceedingly important when considering treatment methods for termite infestations. Colony formation through budding or extension of an existing colony allows for the infested area to be quite large and consisting of a great number of individuals. Although termite infestations in the state of Wisconsin tend to be patchy and isolated, the use of a baiting system to remove the termites from a structure may require more baits and materials than it would if baiting were done in another part of the country, or with a different genus of termite. Also, understanding the timing of differentiation and the periods of increased feeding may give insight into a more comprehensive treatment program for dealing with termite eradication programs.

This study also confirms that weekly collections and experimental set-ups effectively reflected the reproduction, growth, and differentiation during the seasonal active periods of a mature population. It shows that monthly or longer collection periods would not adequately reflect the highly variable results in terms of reproduction, survival, growth, and differentiation. Furthermore, it relates to the impact of length of intermolting periods on the changing conditions in the population. Finally, it reflects the necessity for an in-depth consideration of any proposed laboratory termite experiment as to seasonal variation and peak feeding (Arango *et al.* 2006).

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