Journal of Insect Science, (2022) 22(1): 20; 1–6
https://doi.org/10.1093/jisesa/ieac002
Research

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The Life Span and Levels of Oxidative Stress in Foragers Between Feral and Managed Honey Bee Colonies

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Subject Editor: Michael Simone-Finstrom

Received 16 May 2021; Editorial decision 4 January 2022

Abstract

Molecular damage caused by oxidative stress may lead to organismal aging and result in acute mortality to organisms. Thus, oxidative stress resistance and longevity are closely linked. Honey bees (*Apis mellifera*) are the most important managed pollinator in agriculture, but the long-term survival of honey bees is seriously threatened. Feral honey bee colonies can be used as natural resources to improve honey bee health. One question we ask here is whether feral honey bees are stress resistant or survive longer than managed bee populations. More work is needed to determine the impact of oxidative stress on honey bee health and survival. In this study, we used paired colony designs to compare the life span of worker bees (foragers) between feral and managed colonies and their levels of oxidative stress. Each pair of colonies shared similar foraging resources. The results indicated that foragers in feral colonies had longer survival times and life spans than those in managed colonies. The levels of oxidative stress from lipid damage content in feral colonies were higher than those in managed colonies, indicating that they used a tolerance mechanism rather than a repair mechanism to survive. Our study provides new insights into a colony difference in the physiology and oxidative stress resistance of feral honey bees compared with managed colony stocks.

Key words: pollinator, survival, feral, lipid damage, protein damage

Oxidative stress is a redox-sensitive phenomenon that occurs when reactive oxygen species (ROS) accumulate in a living system faster than the detoxification rate of the organism (Farooqui and Farooqui 2012, Farooqui 2014, Li-Byarlay and Cleare 2020). Reactive oxygen species include peroxyl radicals, hydroxyl radicals, hydrogen peroxides, and superoxide anions. Levels of ROS that exceed the capacity of antioxidant defenses, such as detoxifying enzymes and free radical-scavenging molecules, cause the lipid peroxidation of cell membranes, the cross-linking of proteins, DNA fragmentation and damage, and potential cell death (Johnson et al. 2002, Arking 2006, Li et al. 2008, Bryden et al. 2013, Li-Byarlay and Cleare 2020).

Pollinators are critical players in a sustainable ecological system because they fertilize a substantial number of plants and crops (Kremen et al. 2002, Aizen and Harder 2009, Hunt et al. 2016, Burkle et al. 2017, Isaacs et al. 2017). The honey bee is the most important managed pollinator in the production of crops and fresh produce (Bond et al. 2014). However, honey bee colony populations are in a steady decline of 30–40% annually, especially in the managed honey bee industry (i.e., commercial beekeeping; Kulhanek et al. 2017). Maintaining a sustainable beekeeping industry is crucial for agriculture, given the increasing demands for pollination as populations of wild pollinator species continue to decline (Aizen and Harder 2009, Potts et al. 2010). Factors causing the continued decline of honey bee populations are heterogeneous (Neumann and Carreck 2010) and include pathogens, pesticides, nutrition, genetic variability, and management styles (Nazzi and Le Conte 2016).

Among and within species, substantial variation exists in ROS susceptibility and longevity. However, the mechanisms underlying this variation have not been elucidated. Specifically, survival advantages during an acute oxidative stress event or the normal life history could be conferred by prevention, repair, or tolerance of the molecular damage (Guzmán-Novoa et al. 1994; Rueppell et al. 2007; Li-Byarlay et al. 2016; Taric et al., 2019, 2020; Li-Byarlay and Cleare 2020). Feral or wild honey bee colonies live in a nonmanaged environment and are under natural selection. In this environment, *Varroa destructor* mites persist, and the bees are equipped with greater immunocompetence (Seeley 2007, Locke and Fries 2011, Tarpy et al. 2015, López-Uribe et al. 2017). Previous reports showed that colonies living in traditional trmka hives, similar to feral colonies, were free of several major bee pathogens and viruses (Taric et al. 2019). A different environment, such as migratory beekeeping management, is known to affect the life span of and oxidative stress in honey bees (Simone-Finstrom et al. 2016, Li-Byarlay and Cleare 2020). Previous research on honey bee survival in a laboratory bioassay showed a

significant difference in life spans between honey bee colonies collected in a low-urbanization environment versus a high-urbanization environment (Youngsteadt et al. 2015). However, oxidative stress levels and aging between feral and managed colonies are unknown. Also unknown is whether such differences may be due to different management styles, different living environments, or different landscapes and natural resources. The question addressed by the present study was whether feral colonies display different life spans or different levels of oxidative stress compared with managed colonies. Research on bee survival and oxidative stress will contribute a valuable explanation of the physiological difference between feral and managed honey bee colonies.

Materials and Methods

Bee Population and Sample Collection

A paired colony design was carried out by matching a feral colony with a managed colony within a 3.2 km (2 mi) radius (Fig. 1). Three paired feral and managed colonies were sampled in 2018 (Table 1). Feral colonies were in a natural habitat of tree cavities and were paired with a managed colony within the 3.2 km (2 mi) range. All the feral colonies had survived naturally for at least two winters before our collection time, according to nearby beekeepers and our own observations. The managed colonies were all originally purchased as commercial package bees in 2018 and maintained by beekeepers following normal beekeeping practices. All managed colonies were provided with drawn-out empty combs and foundations.

For each colony, returning pollen foragers were collected at the hive entrance with a heavy-duty handheld aspirator (catalog no. 2820GA, BioQuip, Rancho Dominguez, CA) in midmornings (10:00 a.m.–12:00 p.m.), and bees were kept in an insect-collecting chamber with an end cap (catalog no. 2820D, BioQuip) with honey. The estimated age of returning pollen foragers was approximately 18 days, based on previous reports (Pankiw and Page 2000). Other reports also showed that the life span of foragers in feral colonies can be studied in both field and lab settings (Rueppell et al. 2007, Appler et al. 2015, Hinshaw et al. 2021). Live foragers were brought back to the laboratory and kept in a Precision high-performance gravity incubator (catalog no. PR205075G-Q540177, Fisher Scientific, Waltham, MA) for survival analysis. At the same time, an additional group of pollen foragers was flash-frozen in the field with liquid nitrogen and kept on dry ice until return to the laboratory, where they were stored in a −80°C freezer until analysis for oxidative stress.

Experiments for the Aging Study and Survival Analysis

The life spans of foragers from the feral colonies and managed colonies were determined under controlled conditions as described previously (Simone-Finstrom et al. 2016). The life span analysis was conducted in August 2018. For each colony type, a group of 20–30 foragers was contained in a 591.5-ml (20 oz.) plastic cup with adequate ventilation in an incubator at 34°C and 50–60% humidity as described previously (Evans et al., 2009). A 5-ml syringe was used as a feeder to provide a 50% sucrose solution when needed.

Experiments for Oxidative Stress

The procedures for the lipid and protein assay with the head and thorax tissues, respectively, were the same as described previously (Li-Byarlay et al. 2016, Simone-Finstrom et al. 2016). In brief, the oxidative damage of lipids was quantified by measuring the malondialdehyde (MDA) level in individual heads. Both the TBARS and the Pierce BCA protein assay kits (Thermo Scientific, Waltham, MA) were used according to the manufacturers' recommendations. Total soluble protein was determined by the BCA protein assay and

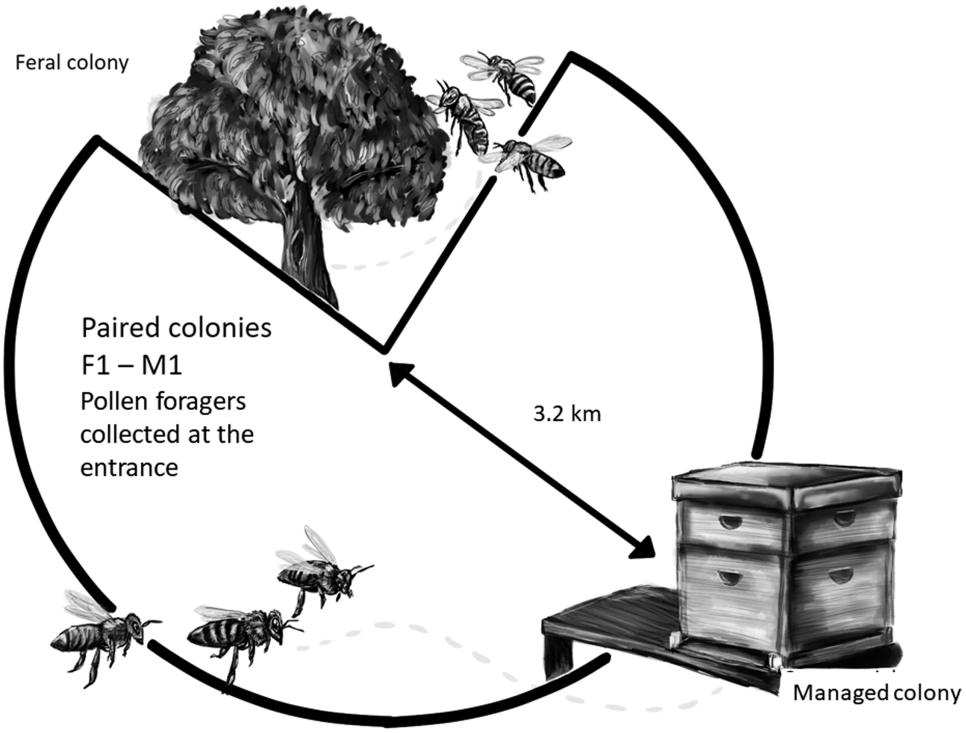

Fig. 1. A paired colony design was carried out by matching a feral colony (F) with a managed colony (M) within a 3.2 km (2 mile) radius. Managed colonies were maintained by beekeepers following normal beekeeping practices. Feral colonies were in the natural habitat of tree cavities.

used to normalize the corresponding TBARS amounts. Each colony type (feral or managed) included at least 12 individual bees. Thirty-nine foragers from feral colonies and another 39 foragers from managed colonies were tested for the TBARS assay.

For the protein carbonyl assay, an OxiSelect protein carbonyl fluorometric assay kit (no. STA-307, CellBiolabs Inc., San Diego, CA) was used to measure the protein carbonyls in thorax samples. A BioTek Synergy LX multimode reader (BioTek, Winooski, VT) was used for fluorescence.

Data Analyses

Differences between feral and package colonies in the life span of foragers were analyzed by the Kaplan–Meier method (a nonparametric survival analysis) with JMP Pro 16 software, with feral versus managed colony treatments as factors in a general linear model. A log-rank test was also performed in the survival analysis (Table 1) to test the null hypothesis that there was no difference in survival between the feral and managed colonies.

Data on oxidative stress were analyzed by using a one-way ANOVA to examine whether differences in oxidative stress (level of MDA or protein carbonyl) were due to the population (feral vs managed bees). Colony and location were considered random effects in the model. Tukey–Kramer post hoc tests were used to make pairwise comparisons of the different experimental groups. Differences were considered significant at $\alpha \leq 0.05$. The normality of the two data sets was tested by using the Kolmogorov–Smirnov test of normality. The Kolmogorov–Smirnov test statistics for the TBARS assay and the protein carbonyl assay were $D = 0.09$ and 0.12, with P-values of 0.43 and 0.22, respectively. Therefore, our data were not significantly different from normal distributions.

Results

Survival Analysis

To compare colonies, we identified at least three feral colonies in three different locations in Ohio. For the survival analysis, a log-rank test was performed to provide a statistical comparison of the two groups, feral versus managed colonies (Table 1). Our data indicated that the life span of feral bees was significantly longer than that of managed bees (log-rank test for pairs 1, 2, and 3: $z = 4.28$, 4.12, and 2.65; $P < 0.001$, $P < 0.001$, and $P = 0.008$; Table 1).

To investigate the difference in life span between the feral and managed package colonies, we performed a Kaplan–Meier test. For the feral colonies, survival ranged from 47 to 57 days. In contrast, the managed colonies displayed a shorter life span of 28 to 42 days (Fig. 2).

Lipid Damage Between the Feral and Managed Colonies

The results in Fig. 3A show a significant difference in lipid damage between the two colony types. The feral colonies tested displayed a higher level of MDA than did the managed colonies ($F_{1,76} = 5.07$, $P = 0.027$). The results in Fig. 3B demonstrate the level of oxidative stress in lipid damage among the three different locations. Paired colonies in the location of Cedarville (Ce) showed the most distinguishing patterns (Fig. 3B).

Table 1. Bee colony information from the aging and oxidative stress tests between feral (F) and managed (M) bees in Ohio

Site	*Z*	*P*-value	Colony name	Feral colony GPS location	Managed colony GPS location	Date of collection
Fairborn (Fa)	4.28	<0.001	F1(F-Fa), M1(M-Fa)	39.8412864, −84.0175809	39.8049291, −84.0599239	July 3, 2018
Cedarville (Ce)	4.12	<0.001	F2(F-Ce), M2(M-Ce)	39.7469314, −83.8174640	39.7469314, −83.8174640	Aug 23, 2018
Bellefontaine (Be)	2.65	0.008	F3(F-Be), M3(M-Be)	40.395217, −83.620033	40.36860, −83.62281	July 2, 2018

A log-rank test was performed in the survival analysis.

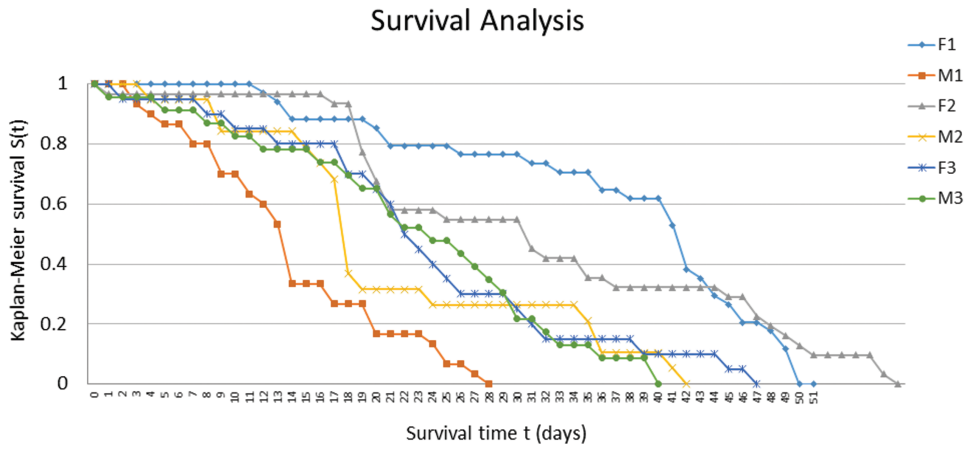

Fig. 2. A Kaplan–Meier plot for six groups associated with colony survival, three feral (F1,2,3) and three managed colonies (M1,2,3). The paired colonies (F1–M1, F2–M2, and F3–M3) were within a 3.2 km (2 mi) radius. The life span of bees in the feral colonies was significantly longer than that of bees in the managed colonies (Kaplan–Meier survival test S(t), $P < 0.001$). The x-axis shows the life span in days, and the y-axis shows the percentage of survival (0–100%). $N = 120$. Individuals were collected per colony ($\chi^2 = 6.48$, $P < 0.001$).

Protein Damage Between the Feral and Managed Colonies

No significant difference was found in protein damage levels between the feral and managed colony types, as illustrated in Fig. 4A ($F_{1,72}$ = 3.01, P > 0.05). The results in Fig. 4B demonstrate the levels of protein carbonyl, a marker of oxidative stress, detected among the three different locations. All three locations showed similar patterns.

Discussion

The aim of this study was to compare the survival and levels of oxidative stress between feral and managed honey bee colonies. The main finding of the study was that the life span of foragers from feral honey bee colonies was longer than that of foragers from managed colonies. Previous findings on the survival of feral bees compared with managed bees showed a similar life span over a short period of time (Youngsteadt et al. 2015). After a swarm chooses where to land in a natural habitat, the wild honey bees in feral colonies live in the tree cavities (Seeley 2007). Thus, feral colonies can serve as a resource to study the fundamental physiology and ecology of honey bees.

In addition, our data on the levels of oxidative stress between feral and managed colonies revealed that levels of lipid damage and protein oxidation in feral colonies were higher than those in managed colonies. These data indicated that feral and managed honey bees had different abilities to deal with oxidative stress. A high level of oxidative stress and a long life span indicate that feral bees may utilize a tolerance mechanism, rather than a repair mechanism, to deal with oxidative stress. Our results provide a basic knowledge of the oxidative stress in wild honey bee populations, in addition to their genetic diversity and immunocompetence (Seeley 2007, Morimoto et al. 2011, Tarpy et al. 2015, López-Uribe et al. 2017). Additionally, the results support our hypothesis that these oxidative stress levels differ between the feral and managed bee colonies. Only a few studies have shown different MDA levels between commercial and traditional colonies in trmka hives without human manipulation, similar to feral colony conditions (Taric et al. 2020), which may be an indication of anthropogenic influence or manipulation. The data presented here provide unique information on the life span and oxidative stress of foragers among feral honey bees.

Not much is known about the protein damage between feral colonies compared with managed colonies. Our results showed no significant difference between feral and managed colonies in protein carbonyl damage in foragers. The level of protein damage in the tested foragers was similar to that of 2-day-old young nurse bees treated with selenate or selenite in another report (Alburaki

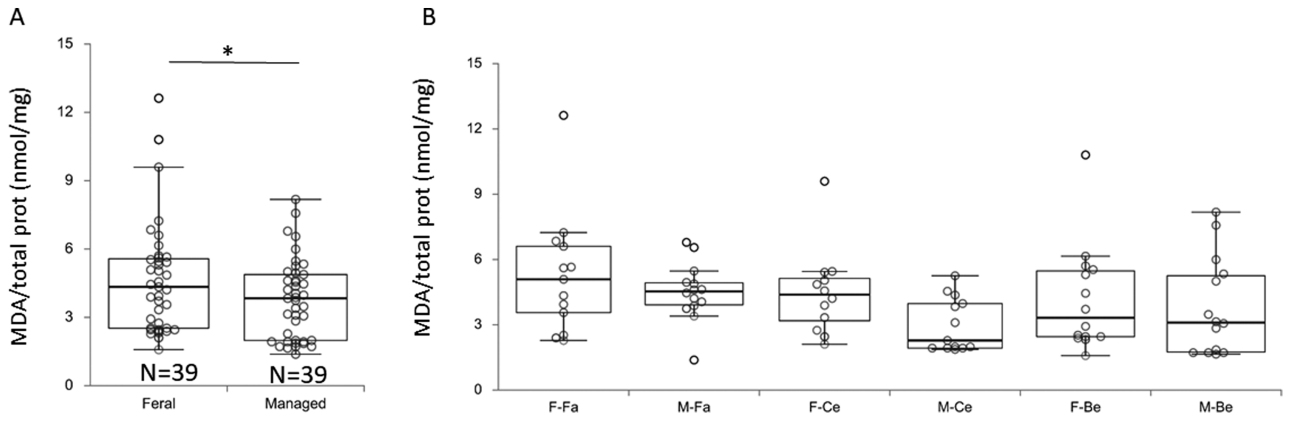

Fig. 3. (A) Comparison of the oxidative stress biomarker malondialdehyde (MDA) between feral and managed colonies. Numbers below the box plots indicate the total number of bees tested. P = 0.027. (B) Boxplots to show the levels of lipid damage from each location (Fa, Fairborn; Ce, Cedarville; Be, Bellefontaine) as paired colonies (F, feral; M, managed).

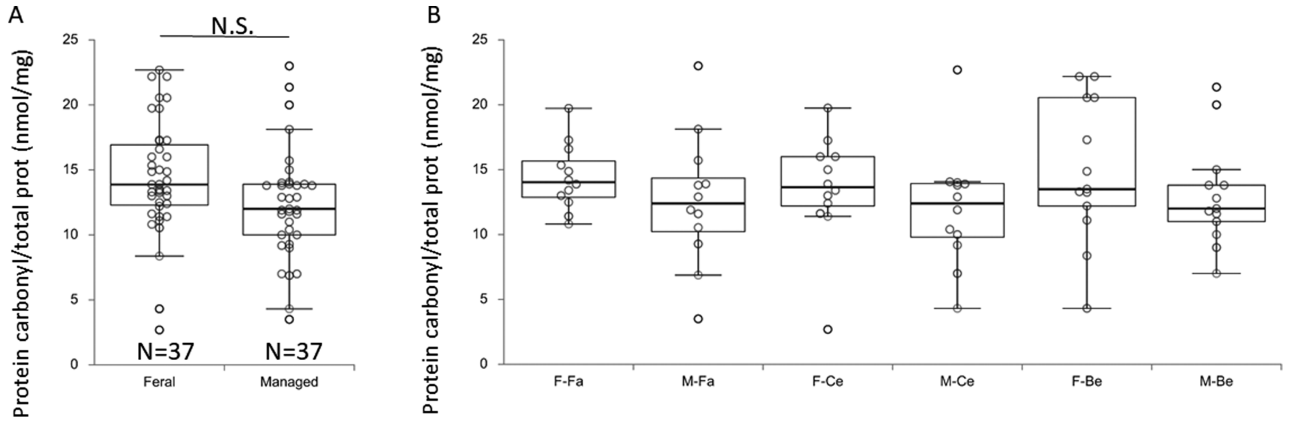

Fig. 4. (A) Comparison of the protein carbonyl levels in box plots between feral and managed colonies. The numbers below indicate the total number of bees tested for each category. P > 0.05 (N.S., nonsignificant). (B) Boxplots to show the levels of protein damage from each location (Fa, Fairborn; Ce, Cedarville; Be, Bellefontaine) as paired colonies (F, feral; M, managed).

et al. 2019). The trend of slightly high levels of protein damage in our data indicated that foragers in feral colony may experience a slightly higher level of ROS activity. The change in protein carbonyl followed a similar trend as the data shown for lipid damage. One potential explanation is that foragers from feral colonies may exhibit physiological tolerance for oxidative stress on lipid, as shown in a previous study of honey bee drones (Li-Byarlay et al. 2016). In future research, we hope to further reveal the molecular mechanism underlying the potentially greater tolerance of feral bees.

Previous research has indicated that feral honey bees experience different parasitic pressure than managed honey bees (Thompson et al. 2014, Hinshaw et al. 2021) and greater immunocompetency (Appler et al. 2015, López-Uribe et al. 2017). Williams et al. (2019) found a lower infection rate of the trypanosome parasite *Lotmaria passim* (16%) in feral colonies than in managed colonies. Taric et al. (2019) also reported higher pathogen loads in commercial colonies compared with traditional colonies in conditions similar to feral conditions. In general, high pathogen infections can reduce the life span of honey bee colonies (Goblirsch et al. 2013).

Honey bees can show varied responses to oxidative stress, depending on their current life stage. This research study tested forager bees as the later adult stage, similar to a previous study by Seehuus et al. (2006). It is common to collect foragers to test physiological questions in feral or wild colonies (Seeley 2007, Appler et al. 2015, Hinshaw et al. 2021). However, we were unable to collect nurse bees or young brood for further testing because of our limited access to feral or wild colonies. Foragers display an increased oxidative capacity compared with nurse bees because of the energy demands of flight (Harrison 1986, Williams et al. 2008, Margotta et al. 2018).

Both feralization and domestication can change the selection pressure and population dynamics of bee colonies (Gering et al. 2019). Phenotype variations between feral and managed colonies are due to natural selection versus artificial selection (Parker et al. 2010). For example, Le Conte et al. (2007) showed that feral bee populations that survived *Varroa* mites without miticide treatment or human interference had lower honey production compared with treated commercial or managed colonies. A review by Locke (2016) summarized research on how honey bee colonies, including feral colonies, survive *Varroa* mites in a natural setting. *Varroa destructor* genotype traits are an influence when investigating the oxidative stress and mite pressure on colonies (Anderson and Trueman 2000).

In summary, our study showed that compared with managed colonies, foragers from feral colonies displayed longer life spans and at the same time showed greater oxidative stress, as expressed through lipid damage but not protein carbonyl damage. This study provided new evidence of the aging and state of oxidative stress of feral honey bee colonies, illustrating the importance of studying feral honey bees for bee health.

Acknowledgments

We thank Brad Armstead, Dwight Wells, and Phil Young for field assistance in sample collection, Ashley Cordle for graphic design, and Susan Krusemark for the assistance of editing.

Funding

Financial support was granted to H.L-.B. by the United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA) Evans-Allen fund (NI171445XXXXG004, NI201445XXXXG018-0001), a USDA Sustainable Agriculture Research and Education North-Central Region Partnership Grant (ONC19-062) and Research Education Grant (LNC21-459), a USDA Agriculture and Food Research Initiative Seed Grant (2020-67014-31557), and a USDA Capacity Building Grant (2021-38821-34576). K.W. was supported by Central State University. X.C. was supported by the USDA NIFA Evans-Allen.

Author Contributions

H.L.B.: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-review & editing. K.W., X.C.: Data curation; Methodology; Formal analysis; Writing—original draft

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