Effects of Long-Term Exposure to Electrostatic Radiation on the Mean Relative Growth Rate and Feeding Behavior of *Sitobion avenae* (Hemiptera: Aphididae)[1]

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Abstract Previous research has indicated that electrostatic radiation may induce biological and physiologic changes in plants and aphids. This study was conducted to determine effects of long-term exposure of electrostatic radiation on biology and feeding behavior of *Sitobion avenae* (F.). To imitate the natural electrostatic radiation stress, both wheat seeds and *S. avenae* nymphs (produced within 12 h) were exposed to an electrostatic radiation of 4 kV/cm for 20 min and 5 kV/cm intensity for 40 min, respectively. Controls received no treatment (0 kV/cm). Then, the mean relative growth rate (MRGR) and feeding behavior of the 5th-, 10th-, 15th-, 20th-, 27th-, 33rd-, and 39th-generation aphids were examined, respectively. Data showed that electrostatic radiation exposure within a certain period of time can promote weight increases of *S. avenae*, but long-term exposure of electrostatic radiation had an adverse effect on the weight gain of aphids. The comparison of the two treatment intensities showed that electrostatic radiation at the intensity of 4 kV/cm for 20 min had a significant influence on the probing behavior of *S. avenae*. This study initially evaluated the effect of electrostatic radiation on *S. avenae* from the aspect of nutrient feeding behavior, which further verifies the biological characteristics of electrostatic radiation.

Key Words electrostatic radiation, *Sitobion avenae*, mean relative growth rate (MRGR), EPG, feeding behavior

The earth, as a negative electric body, forms an atmospheric electrostatic field of about 130 V/m between the positive ionosphere at the top of the atmosphere and on the surface of the earth. Life on earth has been constantly evolving in this electrostatic field (Wu et al. 2005). There are certain charged substances in the living body that are closely related to their growth and development (Zhang 1990). In recent years, with the advancements of science and technology, constructions of subways, trams, and high-voltage direct current (DC) wires are increasing. These facilities are bound to generate electrostatic fields. Consequently, biological effects caused by electrostatic radiation has attracted the attention of researchers (Schmiedchen et al. 2018). *Sitobion avenae* (F.), as a piercing-sucking herbivore and an R-strategist, is not only an important pest on wheat, but also has the characteristics of small body size, high reproduction rate, and short life cycle for

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general ecological studies (Luo et al. 2017, Zhang et al. 2017, 2018). Thus, when the external conditions change, the aphids are exposed to any given environmental stresses. These characteristics further testify that *S. avenae* is an ideal research subject to elucidate the biological effects of insects under different stress, such as electrostatic radiation (Luo et al 2016, Luo et al. 2018).

Studies on biological effects of electrostatic radiation on insects mainly included two fields: behavior and reproduction (Edwards 1961, Jackson et al. 2011, Maw 1961, Newland et al. 2008, 2015, Perumpral et al. 1978). Previous research shows that the developmental period of *S. avenae* could be affected either the aphids are directly exposed to electrostatic radiation or aphids feed on wheat seedlings that developed from seed treated by electrostatic radiation or when both aphids and wheat seeds are both exposed to electrostatic radiation (He et al. 2016, Li et al. 2016, Luo et al. 2016). However, the effects of electrostatic radiation on the behavior of aphids are not well understood to date.

All living organisms (both host plants and aphids) are directly exposed to electrostatic radiation in the nature. Previous studies also have shown that when exposed to electrostatic radiation, the content of cofactors of important elements (such as Fe, Zn, Al) in wheat leaves increased, and it was speculated that changes in the activity of these enzymes (superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) might have negatively affected the structure and physiologic function of wheat leaves (Murr 1964, 1966a, b). Other studies demonstrated that treatment with electrostatic radiation could change the amino acid content, mineral element content, and nonreducing sugar content in wheat seedlings (Wang et al. 1991). Meanwhile, research showed that electrostatic radiation-treated fruits had better freshness and less weight loss after 3-wk storage compared with the control (Palanimuthu et al. 2009). Studies have showed that electrostatic radiation could effectively control pests such as cucumber pests in the field (Zhu et al. 2011). Our focus in this study was to determine whether nutritional changes in wheat after exposure to electrostatic radiation affect the feeding behavior of *S. avenae*, a pest of wheat. Therefore, in this study, we set up a research system to examine whether the electrostatic radiation treatments have long-term effects on insects that could be maintained for multiple generations. To examine aphid feeding behavior, the electronic penetration graph (EPG) technique was used to quantify the long-term effect documented by pattern changes in the EPG waveforms with treatments one time per generation.

The EPG is an electrical physiologic technology used to study the feeding behavior of piercing-sucking insects. It can transform the electrophysiologic pathway of aphid probing into visualized electrical signal waveform and accurately locate the position of insect stylet in host tissue (Hu et al. 2006, McLean and Kinsey 1964, Tjallingii 1978, 1995). After more than 50 yr of development and improvement, the EPG has become a useful tool for analyzing, quantifying, and comparing the feeding behavior of piercing-sucking insects ingesting substances from both xylem and phloem tissues of host plants (Sandanayaka et al. 2014, 2017). It is also widely used to study feeding behavior of piercing-sucking insects along with plant resistance and defense mechanisms (Caillaud et al. 2010). The mean relative growth rate (MRGR), as a simple and easy-to-measure indicator, can well reflect the nutritional and physiological changes of *S. avenae* after treatment with electrostatic radiation stress (Hu et al. 2009, Li et al. 2007). The findings of this study are expected to provide reference for plant–herbivore interactions under electrostatic radiation stress.

Materials and Methods

Insect resource. Aphids used in the experiments were the progeny of a single apterous *S. avenae* collected from a field of wheat near Yangling (N 34°17′, E 108°04′), China. The aphid was reared on the host plant wheat cv. Aikang 58 for three to four generations in an environmentally controlled chamber (diurnal temperature of 22 ± 1°C, 60 ± 10% relative humidity, and photoperiod of 16 h light:8 h dark) to obtain the monoclonal population for subsequent experiments (Luo et al. 2020). Fresh wheat seedlings as a food source were replaced each week.

Physical treatments. Previous research demonstrated that electrostatic radiation with a strength of 4 kV/cm for 20 min had the greatest impact on life-table parameters of aphids. Further reduction of the gradient of the treatment dose showed that the effect of a strength of 5 kV/cm for 40 min was similar to that of a strength of 4 kV/cm for 20 min (Luo et al 2016). Thus, two different intensities of electrostatic radiation (4 kV/cm for 20 min, 5 kV/cm for 40 min) were used as long-term exposures. For each treatment, more than 100 wheat seeds of cultivar cv. Aikang 58 with similar sizes and moisture content were exposed to these two intensities. The electrostatic radiation was produced by an electrostatic radiation generator (Lishui Agriculture Development Centre, Lishui China) consisting of two parallel, rectangular aluminum plates (60×25 cm^2) spaced 8.0 cm apart. Wheat seeds were placed in a Petri dish (without cover; 8.0-cm diameter) between the two electrode plates of the electrostatic radiation generator. No electrostatic radiation exposure was used as the control (0 kV/cm). The seeds were then surface sterilized by soaking them in a 1% H_2O_2 solution for 24 h before germination. Finally, the seeds were cultivated in plastic pots ($9 \times 9 \times 10$ cm^3) filled with a soil mixture (organic matter: soil: sand, 1 v:1 v:1 v) and grown in a climate chamber with the conditions described previously. Seedlings were watered quantitatively according to their growth needs. Plants were used in the experiments to rear aphid populations after they grew to the two- to three-leaf stage (12–13 d after germination).

For each treatment, more than 100 *S. avenae* nymphs produced within 12 h were placed in Petri dishes (without cover; diameter, 8.0 cm) and directly exposed to electrostatic radiation of 4 kV/cm for 20 min and 5 kV/cm for 40 min (once every generation). After the treatment, the aphids were reared on the wheat seedlings with the corresponding treatment intensity and grown in the greenhouse conditions as described previously. No electrostatic radiation exposure was used as the control (0 kV/cm). The nymphs treated for the first time were recorded as the F_1 generation. The nymphs born within 12 h after the birth of the F_1 generation adults from each treatment group were exposed to electrostatic radiation again and recorded as the F_2 generation. In different treatment groups, each generation of its descendants has undergone such processing.

Mean relative growth rate. Aphids born within 12 h in the 5th, 10th, 15th, 20th, 27th, 33rd, and 39th generations were weighed individually after the electrostatic radiation treatment and recorded as W_1. Then, every nymph was transferred to and confined individually on a 14-d-old wheat seedling. All wheat seedlings were grown in plastic pots (6-cm diameter) and covered with cylindrical ($6 \times 6 \times 15$ cm^3) transparent plastic Polyethylene terephthalate film. The top was sealed with a nylon gauze to prevent the aphid from escaping and to maintain ventilation. Growth and development of the aphids were observed twice a day. The weight of the adult after the last molt was recorded as W_2. The time from first instar to adult was recorded as development time (DT). Each processing group was repeated 60 times with 60 aphids. Then MRGR was calculated as MRGR $= \ln W_2 - \ln W_1)$/DT.

Feeding behavior of *S. avenae*. The feeding behavior of aphids was evaluated by using a four-channel DC-EPG device (Wageningen University, The Netherlands, input resistance is 1 GOhm). In the 5th, 10th, 15th, 20th, 27th, 33rd, and 39th generations, apterous aphids of the same size and robustness in each treatment group on the third day after becoming an adult were selected for the EPG experiment. The dorsum of aphid was fastened to 2- to 3-cm-long gold wire (diameter, 20 μm) with water-based silver glue. The other end of the gold wire was attached to a 2-cm-long copper wire (diameter, 0.2 mm) soldered to a brass insect pin. The plant electrode was inserted into the soil of the potted wheat seedlings. Aphids were placed on the abaxial side of a leaf, which was nearly fully expanded. The system was placed in a Faraday cage to prevent external environmental interference. Recording was started after a 1-h starvation treatment and recorded continuously for 8 h. The experimental environment was consistent with the conditions of the environmental chamber. Each aphid and wheat plant were used for only one recording with 20 aphids used for each treatment (20 replications).

Statistical analysis. The original waveform obtained in the EPG test needed to use stylet+a_v01.21 software for classification. Then, the EPG automatic analysis software Probe4.3 was used to further classify and analyze the different behavior waveform parameters (Tjallingii 1988). The EPG recorded waveform and MRGR data were subjected to one-way analysis of variance after validation of the normal distribution of the dependent variable in the study. Student-Newman-Keuls tests were used for multiple comparisons among different treatments. The compared parameters were considered statistically significant when $P < 0.05$.

Results

Treatment effects on the MRGR parameter of *S. avenae*. After long-term treatment with electrostatic radiation, the MRGR of each generation of *S. avenae* was changed as shown in Table 1. According to the MRGR parameters, there are significant differences among different treatments in the 20th, 33rd, and 39th generation ($F = 3.34$, df $= 2{,}178$, $P = 0.038$; $F = 5.25$, df $= 2{,}178$, $P = 0.006$; and $F = 11.86$, df $= 2{,}178$, $P < 0.001$; respectively). When exposure to electrostatic radiation lasted for 15 generations, the weight difference and developmental duration of aphids in the treated groups were significantly higher than those in the control group, whereas the MRGR was not significantly different from the control group. When treated under electrostatic radiation until the 20th generation, the MRGR of the *S. avenae* showed an increased trend with the increase of treatment intensity, and there was a significant difference between the 5 kV/cm for 40 min treatment group and the control group. The data analysis of the MRGR and developmental duration showed that it was because of significantly shortened developmental duration in the 20th generation at 5 kV/cm for 40 min. Although the weight difference of *S. avenae* was significantly higher than that of the control group under the 4 kV/cm for 20 min treatment intensity in the 20th generation, the developmental duration and MRGR were not significantly different from the control. When exposed to electrostatic radiation until the 33rd generation, the MRGR of both treatment groups was significantly higher than that of the control group, and the developmental duration was significantly shortened compared with the control. When *S. avenae* was maintained to the 39th generation under long-term electrostatic radiation, the weight difference and MRGR of the 4 kV/cm for 20 min treatment group were reduced, and the developmental duration was significantly prolonged.

From the perspective of changes in different generations, the weight difference and MRGR in the two treatment groups increased first and then decreased in the later generations with continued treatment once per generation. Developmental times show a trend of initially decreasing, then increased, and decreased again, and finally increasing in later generations at 4 kV/cm for 20 min. In particular, the developmental duration was significantly prolonged in the 15th, 20th, and 39th generations, whereas it was the shortest in the 33rd generation. Under the treatment intensity of 5 kV/cm for 40 min, the developmental duration of *S. avenae* show an initial increase, then a decrease, and increased again, and finally decreased in later generations, which was significantly prolonged in the 15th generation and was the shortest in the 20th generation.

Changes of Np wave of aphids under different intensities of electrostatic radiation. The Np wave refers to the process from aphid colonization on the leaves to its initiation of probing, which is the time required to find a suitable piercing site. Statistical analysis on the total duration of Np (Table 2) indicated that the total duration of Np in the treatment groups was significantly lower than the control when the *S. avenae* reached the 10th to 15th generations under the electrostatic radiation treatment ($F = 6.635$, df $= 2{,}42$, $P = 0.003$ and $F = 5.805$, df $= 2{,}42$, $P = 0.006$, respectively). In the 27th generation, the total duration of Np in the treatment group of 4 kV/cm for 20 min was the lowest, and there was significant difference compared with the 5 kV/cm for 40 min treatment ($F = 3.542$, df $= 2{,}42$, $P = 0.041$). Between different generations, the total duration of Np in the treatment groups increased as the generations increased. These results revealed that short-term electrostatic radiation treatment can shorten the time before probing, which is more conducive to the occurrence of aphid feeding behavior. However, total probing times shortened with the later generations under long-term electrostatic radiation treatment. Feed behavior occurred earlier under high-intensity treatment compared with the low-intensity treatments.

Changes of Pd wave of aphids under different intensities of electrostatic radiation. Pd wave refers to the electrical potential difference generated when aphids pierced the cell membrane. This is the path wave of aphid feeding and often accompanied by C waves, which refers to the pathway activities in mesophyll. The variation in Pd value was inconsistent with the treatment intensity increasing in each generation (Table 3). In the fifth generation, the number of Pd was significantly reduced at 4 kV/cm for 20 min compared with 5 kV/cm for 40 min treatment ($F = 4.165$, df $= 2{,}42$, $P = 0.022$). However, while proceeding to the 27th generation, the number of Pd at 4 kV/cm for 20 min was significantly higher than the other two

Table 1. Mean relative growth rate (MRGR), weight difference (Δ lnW), and development duration (DT) of *S. avenae* at two electrostatic radiation treatment levels in succeeding generations.

		Treatment		
Parameters	**Generation**	**CK**	**4 kV/cm for 20 min**	**5 kV/cm for 40 min**
MRGR*	G5	0.3248 ± 0.0094aA	0.3208 ± 0.0101aB	0.3089 ± 0.0108aA
	G10	0.3327 ± 0.0113aA	0.3598 ± 0.0106aC	0.3476 ± 0.0105aB
	G15	0.3363 ± 0.0095aA	0.3456 ± 0.0068aBC	0.3443 ± 0.0065aB
	G20	0.3355 ± 0.0096aA	0.3593 ± 0.0078abC	0.3655 ± 0.0084bBC
	G27	0.3365 ± 0.0101aA	0.3623 ± 0.0100aC	0.3447 ± 0.0105aB
	G33	0.3442 ± 0.0086aA	0.3785 ± 0.0090bC	0.3864 ± 0.0112bC
	G39	0.3440 ± 0.0067bA	0.2883 ± 0.0090aA	0.3334 ± 0.0092bAB
Δ lnW**	G5	2.33 ± 0.04aA	2.39 ± 0.05aB	2.27 ± 0.05aA
	G10	2.45 ± 0.06aA	2.58 ± 0.06aC	2.57 ± 0.05aB
	G15	2.53 ± 0.06aA	2.71 ± 0.04bCD	2.74 ± 0.04bC
	G20	2.52 ± 0.05aA	2.81 ± 0.04bD	2.50 ± 0.05aB
	G27	2.49 ± 0.05aA	2.63 ± 0.05aC	2.51 ± 0.05aB
	G33	2.52 ± 0.04aA	2.65 ± 0.05aC	2.66 ± 0.05aBC
	G39	2.49 ± 0.04cA	2.19 ± 0.05aA	2.32 ± 0.05bA

Table 1. Continued.

Parameters	Generation	Treatment		
		CK	4 kV/cm for 20 min	5 kV/cm for 40 min
DT(d)[†]	G5	7.31 ± 0.12aA	7.65 ± 0.13aBC	7.60 ± 0.16aB
	G10	7.56 ± 0.15aA	7.29 ± 0.11aA	7.54 ± 0.11aB
	G15	7.61 ± 0.09aA	7.91 ± 0.07bC	8.02 ± 0.09bC
	G20	7.70 ± 0.13bA	7.93 ± 0.09bC	6.91 ± 0.07aA
	G27	7.52 ± 0.11aA	7.39 ± 0.11aAB	7.43 ± 0.11aB
	G33	7.41 ± 0.09bA	7.09 ± 0.09aA	7.05 ± 0.12aA
	G39	7.3 ± 0.07aA	7.76 ± 0.11bC	7.08 ± 0.08aA

N = 60.

* Mean ± SE MRGR followed by different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas the different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

** Mean ± SE Δ lnW followed by the different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas the different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

† Mean ± SE DT followed by the different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas the different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

Table 2. Total duration of Np waveform (min) of *S. avenae* at two electrostatic radiation treatment levels in succeeding generations.

	Total Duration of Np (min)*		
Generation	CK	4 kV/cm for 20 min	5 kV/cm for 40 min
G5	128.79 ± 12.50aA	107.13 ± 13.77aA	131.38 ± 15.48aABC
G10	194.50 ± 20.48bA	112.46 ± 10.30aA	147.71 ± 15.51aABC
G15	128.63 ± 12.62bA	82.04 ± 9.84aA	91.30 ± 7.63aA
G20	146.30 ± 14.68aA	131.77 ± 14.94aA	119.91 ± 10.55aAB
G27	140.54 ± 17.02abA	115.27 ± 9.07aA	167.41 ± 16.45bBCD
G33	185.18 ± 19.09aA	190.30 ± 22.06aB	210.23 ± 17.55aD
G39	165.18 ± 17.58aA	193.04 ± 18.90aB	185.65 ± 20.80aCD

N = 15.

* Mean ± SE total duration of Np followed by different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas the different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

groups ($F = 6.630$, df = 2,42, $P = 0.003$). Thus, the value of Pd showed a fluctuation with the growth of generations at 4 kV/cm for 20 min, and the lowest value appears in the fifth generation. There was no significant difference between the 5 kV/cm for 40 min treatment group and the control group. According to the analysis, data for the total duration time of Pd were basically consistent with the changing trend of the number of Pd. However, there was a significant increase in the 27th generation at 4 kV/cm for 20 min, suggesting that electrostatic radiation of 4 kV/cm for 20 min was the critical intensity of the treatment.

Changes of E1 wave of aphids under different intensities of electrostatic radiation. E1 represents the process by which aphids secrete water-soluble saliva into the phloem tissue. With the increase of treatment intensity, the number of E1 increased initially and then decreased in the 15th, 27th, and 39th generations (Table 4; $F = 5.826$, df = 2,42, $P = 0.006$; $F = 9.658$, df = 2,42, $P < 0.001$; and $F = 3.240$, df = 2,42, $P = 0.048$; respectively). Compared with the other groups, it increased remarkably at 4 kV/cm for 20 min, particularly in the 15th and 27th generations. However, the number of E1 waves was significantly reduced at 4 kV/cm for 20 min when exposed to electrostatic radiation for multiple generations and up to the 39th generation. Thus, with increasing generations, the number of E1 in the 4 kV/cm for 20 min treatment group increased and then decreased after the 15th generation. For the 5 kV/cm for 40 min treatment group, the number of E1 also increased at first and then decreased in later generations and reached the highest value in the 20th generation; however, the difference was not significant with the control in each generation throughout the experiment period. The data in Table 4 show a fluctuation in E1: a trend to initially increase, then decrease, and then increase in later generations at 5 kV/cm for 40 min. However, there was no

Table 3. Total duration of Pd (min) and number of Pd of *S. avenae* at two electrostatic radiation treatment levels in succeeding generations.

Parameters	Generations	CK	4 kV/cm for 20 min	5 kV/cm for 40 min
Total duration of Pd (min)*	G5	8.75 ± 0.73aA	12.94 ± 2.12aA	12.62 ± 1.06aA
	G10	13.81 ± 0.84aA	12.44 ± 1.00aB	11.39 ± 1.12aA
	G15	11.18 ± 1.05aA	9.15 ± 1.02aAB	10.18 ± 1.12aA
	G20	10.62 ± 1.28aA	12.18 ± 0.71aAB	10.66 ± 0.99aA
	G27	14.12 ± 1.29aA	10.47 ± 0.78bB	8.85 ± 1.32aA
	G33	11.14 ± 1.12aA	8.45 ± 0.91aAB	8.33 ± 0.78aA
	G39	8.29 ± 0.82aA	9.76 ± 0.70aA	9.38 ± 1.08aA
Number of Pd**	G5	134.46 ± 14.38abA	107.4 ± 9.52aA	160.8 ± 14.69bA
	G10	150.66 ± 12.31aA	188.86 ± 11.65aC	160.93 ± 14.35aA
	G15	145.93 ± 16.40aA	178.6 ± 16.55aBC	162.93 ± 18.54aA
	G20	179.66 ± 10.21aA	157.13 ± 16.57aABC	163.2 ± 14.85aA
	G27	157.00 ± 12.65aA	209.53 ± 18.10bC	124.93 ± 18.37aA
	G33	136.07 ± 13.95aA	173.53 ± 18.65aBC	132.33 ± 12.89aA
	G39	139.66 ± 9.22aA	124.4 ± 13.45aAB	126.66 ± 12.87aA

N = 15.

* Mean ± SE of total duration of Pd followed by different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

** Mean ± SE of number of Pd followed by different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

Table 4. Total duration of E1 (min) and number of E1 of *S. avenae* at two electrostatic radiation treatment levels in succeeding generations.

Parameters	Generations	CK	4 kV/cm for 20 min	5 kV/cm for 40 min
Total duration of E1 (min)*	G5	75.27 ± 21.84aA	118.22 ± 22.12aA	64.91 ± 12.42aAB
	G10	39.04 ± 7.16aA	53.59 ± 11.57aA	38.84 ± 7.99aAB
	G15	114.86 ± 31.53aA	108.05 ± 12.56aA	73.69 ± 13.42aAB
	G20	73.64 ± 12.66aA	58.20 ± 15.93aA	80.99 ± 23.54aB
	G27	76.39 ± 17.23aA	62.67 ± 12.49aA	45.60 ± 16.47aAB
	G33	76.23 ± 20.31aA	71.58 ± 20.78aA	19.18 ± 5.51aA
	G39	35.70 ± 6.81aA	53.04 ± 22.31aA	85.09 ± 15.20aB
Number of E1**	G5	8.53 ± 1.79aA	10.46 ± 1.37aAB	8.53 ± 1.30aAB
	G10	8.26 ± 1.25aA	10.60 ± 1.48aAB	8.33 ± 1.21aAB
	G15	10.40 ± 1.46aA	19.53 ± 2.29bC	13.53 ± 1.91aB
	G20	10.67 ± 1.52aA	12.73 ± 2.01aB	13.73 ± 1.97aB
	G27	9.13 ± 1.31aA	13.8 ± 1.29bB	6.26 ± 1.04aA
	G33	8.20 ± 0.95aA	8.80 ± 1.31aAB	5.00 ± 1.33aA
	G39	6.53 ± 1.12abA	5.26 ± 1.35aA	10.46 ± 1.91bAB

N = 15.

* Mean ± SE of total duration of E1 followed by different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

** Mean ± SE of number of E1 followed by different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

significant effect on the total duration of E1 by changing the treatment intensity of electrostatic radiation.

In summary, the electrostatic radiation treatment of 4 kV/cm for 20 min had the greatest influence on the feeding behavior of the salivation process in the phloem. Specifically, within a certain period of time (e.g., 15th–27th generations), electrostatic radiation treatment of 4 kV/cm for 20 min could increase the frequency of occurrence of phloem E1 wave, but after long-term treatment for multiple generations, especially after 30 generations, it had an inhibitory effect on the generation of E1 waves.

Changes of E2 wave of aphids under different intensities of electrostatic radiation. The E2 wave represents the process by which aphids passively ingest nutrients from the phloem. In the 5th, 15th, and 27th generations, the number of E2 was significantly higher in E2 than the other two groups in the 4 kV/cm for 20 min treatment group (Table 5; $F = 4.605$, df $= 2,42$, $P = 0.016$; $F = 4.821$, df $= 2,42$, $P = 0.013$; and $F = 4.541$, df $= 2,42$, $P = 0.016$; respectively). However, when the electrostatic radiation treatment was performed to the 39th generation, the number of E2 at 5 kV/cm for 40 min treatment increased significantly, which was significantly different from the other two groups ($F = 3.969$, df $= 2,42$, $P = 0.026$). According to the longitudinal analysis of different generations, the number of E2 at 4 kV/cm for 20 min increased initially and then decreased, and reached its maximum in the 15th generation, which was significantly different from other generations. The number of E2 increased and decreased with the growth of generations at 5 kV/cm for 40 min, reaching its maximum in the 20th generation. For the total duration of E2, there was no difference between 4 kV/cm for 20 min treatment group and control in any generation. It should be noted that the total duration of E2 continued to decrease after multiple generations of treatment under the 4 kV/cm for 20 min intensity of electrostatic radiation. In the 5 kV/cm for 40 min treatment group, there was a significant difference in the 20th generation compared with the other two treatment groups. The low-intensity treatment had a great influence on the number of E2 especially before the 33rd generation, whereas the high-intensity treatment intensity would significantly affect the total duration of passively ingesting on the phloem sap within a specified time.

Discussion

Previous studies have examined the biological effects of electrostatic fields for decades and showed that the application of electric fields can cause specific reactions in organisms (Schmiedchen et al. 2018). However, sufficient theoretical analysis is lacking as to the mechanism and chemical processes of the biological effects of electric fields. Only through sufficient experimentation can possible mechanisms be elucidated, for example, imposing certain electric fields on an organism to observe macrobiological effects by measuring certain biochemical indicators (Na and Feng 2003, Wu et al. 2004). To further examine electrostatic radiation biological effects on a living organism, this study used the MRGR (a life table parameter) and the EPG waveforms (feeding behavior parameters) to evaluate the effects of electrostatic radiation on the body weight and feeding behavior of *S. avenae* after exposing aphids to electrostatic radiation across

Table 5. Total duration of E2 (min) and number of E2 of *S. avenae* at two electrostatic radiation treatment levels in succeeding generations.

Parameters	Generations	CK	4 kV/cm for 20 min	5 kV/cm for 40 min
Total duration of E2 (min)*	G5	50.93 ± 14.00aA	83.54 ± 23.13aB	69.93 ± 22.04aA
	G10	39.79 ± 14.94aA	39.96 ± 8.75aAB	46.74 ± 15.67aA
	G15	43.31 ± 9.82aA	49.99 ± 6.62aAB	86.32 ± 22.02aA
	G20	32.58 ± 7.12aA	44.47 ± 12.62aAB	85.52 ± 13.77bA
	G27	61.32 ± 19.08aA	63.69 ± 10.47aAB	59.12 ± 15.82aA
	G33	67.04 ± 16.31aA	21.95 ± 5.30aA	43.27 ± 19.16aA
	G39	49.46 ± 14.78aA	20.47 ± 10.11aA	50.99 ± 16.06aA
Number of E2**	G5	3.6 ± 0.58aA	7.33 ± 1.18bA	3.93 ± 1.01aA
	G10	3.73 ± 0.92aA	5 ± 1.10aA	3.93 ± 0.99aA
	G15	6.86 ± 1.36aA	13.73 ± 2.22bB	7.86 ± 1.31aAB
	G20	6.2 ± 1.21aA	8.33 ± 1.68aA	10.2 ± 1.90aB
	G27	5.4 ± 1.06abA	7.6 ± 0.99bA	3.6 ± 0.72aA
	G33	4.86 ± 0.83aA	5.13 ± 1.08aA	2.66 ± 0.91aA
	G39	3 ± 0.63aA	3 ± 0.99aA	6.66 ± 1.41bAB

N = 15.
* Mean ± SE total duration of E2 followed by different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).
** Mean ± SE number of E2 followed by different lowercase letters in each row indicate significantly different among different doses of electrostatic radiation ($P < 0.05$), whereas different uppercase letters in each column indicate significantly different among different generations ($P < 0.05$).

multiple generations and rearing them on wheat plants grown from seed that was also exposed to electrostatic radiation. The results of these studies revealed that the influence of electrostatic radiation on aphid biology showed different trends with increasing generations of *S. avenae*. Data on weight increase of *S. avenae* indicated that weight initially increased and was then inhibited in later generations of the aphid after treated by electrostatic radiation. Growth promotion was mainly manifested in the weight difference and the MRGR of *S. avenae* being higher than the control group during the 10th–33rd generations in the electrostatic radiation treatment group. Similar studies have shown that the hatching time of *Bombyx mori* L. eggs was prolonged and pupal weight significantly increased after short-term treatment on fresh eggs under electrostatic radiation at 10 kV/cm for 3 min (Ju et al. 2010). Other trials using electrostatic radiation to treat rats have also shown that continuous treatment with an electrostatic radiation of 50 kV/m for 14–28 d can promote increased body weight (Gan et al. 1997). However, in our trials, when treatment with electrostatic radiation was continued until the 39th generation, weight gain of *S. avenae* was inhibited, especially under the treatment intensity of 4 kV/cm for 20 min, which indicated that electrostatic radiation may induce a different effect on different species and the effects may depend on doses of electrostatic radiation.

Changes caused by the biological effect of electrostatic radiation likely affected the feeding behavior of *S. avenae*. Through the analysis of the feeding behavior parameters of many generations of *S. avenae*, the results indicated that the nonprobing time was shortened when *S. avenae* was exposed to electrostatic radiation until the 10th–15th generations. However, the nonprobing time began to increase in later generations. This also demonstrated that short-term electrostatic treatment on *S. avenae* has a promoting effect for feeding behavior, but this promotion effect gradually disappears with the adaptation of aphids studied. This is consistent with weight change as generations increase. In addition, feeding behavior of *S. avenae* in the phloem also showed that, as generations increased, number of probes in the phloem by *S. avenae* first increased and then decreased with the prolongation of generations exposed to electrostatic radiation. This was especially significant at the 4 kV/cm for 20 min treatment. Previous studies have shown that the increase of reactive oxygen species (ROS) in host plants increases the frequency of probing and shortens the phloem feeding time of aphids (Guo et al. 2019). Therefore, it can be speculated that the number of phloem probes within a certain generation in this study may be caused by the large amount of ROS produced in plants. Research on weight gain of *S. avenae* has shown that the treatment of electrostatic radiation in a certain generation has promoted increased weight in *S. avenae*, so it is speculated that the increase of its growth and metabolic rate will force *S. avenae* to have a stronger appetite; hence, probing frequency in phloem increased. Frequent probing inevitably will cause certain mechanical damage to the plant which, in turn, leads to an increase in the active oxygen content in the plant. High levels of ROS in plants can induce plant defenses in the epidermal and mesophyll tissues and activate phytohormone-mediated resistance, cell wall cross-linking, and callus deposition (Jaouannet et al. 2014, Laitinen et al. 2017, Rasool et al. 2017), which further make it difficult for *S. avenae* to reach the sieve element of phloem tissue. Therefore, aphids probe more frequently in the epidermal and mesophyll tissues in a certain period of time, such as the Pd wave. With later generations, electrostatic accumulation begins to adversely affect aphid growth.

Therefore, the demand for aphids feeding on the phloem is reduced; thus, the piercing damage to the plants is reduced. Conversely, the antioxidant enzyme system in plant has a certain scavenging effect on active oxygen, which causes the level of active oxygen in wheat to decrease. For this reason, the frequency of phloem probing is also significantly reduced with later generations of *S. avenae*.

In addition, electrostatic radiation has a dose effect, which includes treatment intensity and treatment time. Dose uncertainty means that different doses of an electric field can produce different biological effects on the same organism (Na and Feng 2003). In a previous study, our team used different electrostatic radiation (3, 4, and 5 kV/cm) and time combinations (20, 40, and 60 min) to treat *S. avenae*, indicating that the treatment intensity of 4 kV/cm for 20 min or 5 kV/cm for 40 min had a great influence on the population growth and respiratory metabolism of *S. avenae*. Based on these two intensities, the current study examined the optimal intensity by investigating the effects of feeding behavior of *S. avenae* directly exposed to electrostatic radiation across multiple generations. The results of the study on feeding behavior indicate that the number and duration of Pd initially increased and then decreased with the increase in generations and reached a maximum in the 27th generation at 4 kV/cm for 20 min. However, the number and duration of Pd waves did not change in later generations, and there was no significant difference between the control and treatment at 5 kV/cm for 40 min. Similarly, compared with the 4 kV/cm for 20 min treatment, the number of phloem feedings at the treatment intensity of 5 kV/cm for 40 min was not different from the control group before the 30th generation.

According to statistical analysis results of various parameters under different treatment intensities, we can draw a conclusion that the electrostatic radiation treatment of 4 kV/cm for 20 min showed a greater impact on feeding activities, which is consistent with our previous report that 4 kV/cm for 20 min is the key intensity to affect development and reproduction of *S. avenae* (Cao et al. 2016, Li et al. 2016, Luo et al. 2019).

In summary, the current study shows that electrostatic radiation treatment within a certain period of time can promote increased weight in *S. avenae*, but continuous treatment of once per generation will have an adverse effect on the weight gain of aphids. Aphid feeding behavior was also affected by the electrostatic radiation treatments by showing increased number of probes in early generations. However, with continuous treatment of once per generation, the total probing time in later generations was reduced, and the amount of probing in mesophyll and phloem also decreased correspondingly. The comparison of the two treatment intensities showed that the electrostatic radiation treatment at the intensity of 4 kV/cm for 20 min has a greater influence on number of probes of *S. avenae*. This study only treated the host plant seeds, and the wheat used for rearing aphids in each generation was consistent. However, continuous treatment with electrostatic radiation on *S. avenae* once per generation could have an accumulative effect on aphid throughout experimental period of 39 generations. Therefore, it could be postulated that the consistent one per generation treatment with the electrostatic radiation treatment mainly affected aphid feeding behavior and obtaining nutrients, which, in turn, causes aphid weight changes. On the other hand, the physiology and behavior of *S. avenae* affects aphid–host plant interactions, which is also further modulated by changes of aphid feeding behavior.

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