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Effects of Forage Corn Silage Preparation on Early-instar Larval Survivability and Egg Hatchability of Fall Armyworms (*Spodoptera frugiperda*)

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Abstract

In this study, we investigated the effects of laboratory-scale silage preparation using a plastic bag and incubation on the early-instar larval viability and egg hatchability of fall armyworms (FAWs) inoculated into corn. In experiments performed to evaluate the effects on larval viability, the impacts of vacuum and anaerobic conditions were assessed as factors expected to influence larval viability after silage preparation. In the experiment that evaluated the effects on egg hatchability, the effects of enclosed fresh corn or fermented corn silage were examined. In the experiment that evaluated the effects of vacuum during silage preparation, no viable larvae were detected in anaerobic conditions, regardless of the length of incubation time (24 h or 14 days). In the experiment that confirmed the effects of carbon dioxide (CO_2) gas during silage preparation, no viable larvae were noted under enclosed CO_2 conditions, regardless of the incubation period (24 h or 14 days). After 3 days of silage preparation, we also found that egg hatchability was 0.05%. These results suggest that forage corn contaminated with FAW has a reduced larval viability rate and egg hatchability in the early stages of silage preparation, suggesting a low chance of FAW spreading from corn silage prepared under airtight conditions.

Discipline: Agricultural Environment
Additional key words: anaerobic condition, carbon dioxide gas, compaction, larval viability, silage storing

Introduction

Fall armyworms (FAWs; *Spodoptera frugiperda*) are a species of moths that belong to the Noctuidae family and originally inhabit the tropical and subtropical regions of North and South America. FAWs can damage >80 species of crops, including poaceous plants, flying over long distances of up to 500 km per generation. FAWs invaded Africa in 2016, resulting in major agricultural damage, particularly to corn. Subsequently, FAW-inhabited areas rapidly expanded to many regions in Asia, including India and China, reaching Australia in 2020 (Food and Agriculture Organization 2021). Continuous outbreaks are expected across various areas, including East Asia. Despite reduced risk in most regions in Japan due to low temperatures during winter, frequent invasion from southern China is predicted in Kyushu, Shikoku, and Western Honshu every year (Ma et al. 2019). In 2018, FAW-induced damage to corn reached a maximum of approximately 17.7 million metric tons in 12 African countries (Pratt et al. 2018). Appropriate pest management is necessary to prevent further damage caused by FAWs. In Japan, after confirming FAW inhabitation in Kagoshima Prefecture in July 2019 (Ministry of Agriculture, Forestry and Fisheries 2020b), the affected area was found to have expanded to Hokkaido in 2020 (Ministry of Agriculture, Forestry and Fisheries 2020c). Most damages reported in Japan were observed

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in forage corn.

The use of pesticides appears to be effective against FAW infestation. However, in forage corn, effective spraying is difficult, except in the early developmental stages, because of the high grass height (>1 m) in the middle and later stages, which limits the access of tractor-mounted boom sprayers in crop fields. Aerial spraying is also considered difficult because of the following reasons: 1) probable pesticide drift to nearby farm fields and 2) difficulty in reaching the effective pesticide concentration in larvae inhabiting the lower parts of plants or within plants.

In Japan, the Ministry of Agriculture, Forestry and Fisheries has instructed to harvest forage corn or plow the field immediately after an FAW infestation uncontrolled by pesticides (Ministry of Agriculture, Forestry and Fisheries 2020a). Harvested immature forage corn can be used as silage material. However, if the corn is harvested during the confirmation of FAW infestation, the larvae and sometimes eggs may inevitably be mixed with the corn. If FAWs are viable after silage preparation, rediffusion is possible after silo opening. Thus, countermeasures are required during silo opening. Conversely, if larval viability and egg hatchability after silage preparation are sufficiently low, there is minimal concern about FAW diffusion after silo opening. However, the effect of corn silage preparation on larva viability and egg hatchability of the contaminating FAW remains to be determined.

This study aimed to clarify the effects of factors that may affect FAW's early-instar larval viability and egg hatchability after corn silage preparation under experimental conditions. We artificially inoculated FAW larvae or egg masses to the silage material and prepared laboratory-scale silage using vacuum packs. Then, the survival rate of the larvae and the hatchability of eggs after incubation were assessed.

Materials and methods

All data are presented as mean ± standard deviation unless otherwise stated.

Forage corn used as silage material was cultivated at a field in Kyushu Okinawa Agricultural Research Center (Koshi City, Kumamoto Prefecture).

FAW feeding was performed in an indoor isolated area at Kyushu Okinawa Agricultural Research Center. Larvae were fed artificial feed (Insecta LFS, Nosan Corporation, Yokohama, Japan) after they hatched from eggs. Then, used eggs were laid on the same day to adjust the age of the larvae. The larvae were fed until they reach the second-instar stage, after which they were used for various experiments.

The same cutting machine (CX-201, Yamamoto, Tendo, Japan), paper towel (Kim towels 4-fold type, Nippon Paper Crecia, Tokyo, Japan), vacuum packaging machine (V-301, FUJIIMPLUSE, Toyonaka, Japan; ultimate vacuum of pump, −69 kPa), and vacuum packaging plastic bag (Hiryu N-10, Asahi Kasei Pax, Tokyo, Japan; size, 240 mm × 350 mm; thickness, 70 μm; and oxygen transmission rate, 444 mL/m^2·MPa·24 h) were used in all experiments. The FAW second-instar larval viability after vacuum packaging using the vacuum packaging machine with 50 g of shredded corn leaves (cut 47 mm in length using the cutting machine) and one paper towel in the vacuum packaging plastic bag and after 1 h of further incubation at 20°C was 28.3 ± 27.5% (n = 3).

1. Larval survival experiments

(1) Vacuum experiment

Isolated fresh silking-stage corn leaves were shredded using the cutting machine, with a cutting length of 47 mm. The cutting length and use of isolated leaves were determined to easily seek small-sized larvae. Shredded leaves (50 g) were placed in a vacuum packaging plastic bag with one paper towel to prevent larvae from drowning in dew condensation water during incubation. A total of 20 second-instar FAW larvae were inoculated into each bag. To reproduce different silage fermentation states, 10 bags were sealed with vacuum (the vacuumed group) using the vacuum packaging machine and 10 bags were sealed without vacuum (the sealed group). The bags were incubated at 15°C for 24 h. Then, five vacuumed and five nonvacuumed bags were further incubated at 11°C for 14 days after sealing. These temperature conditions were determined to prevent putrefaction of the silage material in the unsealed control treatment group and prevent larval death from starvation by diapause. After incubation, the bags were opened, and all larvae were isolated from their bag contents. As differentiating alive and dead larvae that are not in motion is difficult, larval viability was measured by observing the motility of the larvae under a microscope after 3 days of feeding with artificial feed. To evaluate silage fermentation, 25 g of shredded corn leaves were obtained from each bag. To determine larval viability under aerobic conditions, 20 second-instar FAW larvae were placed in each of the five ventilated plastic containers with 50 g of shredded corn leaves (unsealed group). The unsealed containers were incubated at the same temperature condition as that of the sealed bags for 14 days, and larval viability was measured as described above.

(2) Carbon dioxide experiment

After removing the corn ear, yellow ripe-stage corn leaves and stems were separated. The leaves and stems were separately shredded using the cutting machine with cutting lengths of 47 mm and 19 mm, respectively. From the shredded leaves and stems, 25 g of each was placed with one paper towel in a vacuum packaging plastic bag. A total of 20 second-instar FAW larvae were inoculated in each bag. To investigate the differences between anaerobic and aerobic conditions, 10 bags were sealed with carbon dioxide (CO_2) gas (CO_2 group) blown from a handheld gas cylinder (Kenis, Osaka, Japan), and 10 bags were sealed with ambient air (air group). To reduce the amount of air remaining, bags in the CO_2 group were first sealed as in the air group, and then the corner of the bag was cut off and lightly pressed to release air; finally, a sufficient amount of CO_2 gas was blown in the bag while the bag was set on the sealer, and the bag was sealed immediately after the blowing was stopped. All bags were incubated for 24 h at 15°C. Subsequently, five bags from each group were further incubated at 11°C for 14 days after sealing. To determine larval viability under aerobic conditions, each of the 20 second-instar FAW larvae was placed into one of the five air-ventilated plastic containers with a mixture of shredded corn leaves and stems as in the air or CO_2 groups (control group). The containers were incubated at the same condition as that of the sealed bags for 14 days after sealing. After incubation, the bags were opened, and all larvae were isolated from the bag contents. Larval viability was determined using the abovementioned method. To evaluate silage fermentation, 25 g of the shredded corn was used.

2. Egg hatchability experiment

All egg masses used in this experiment were oviposited on approximately the same day. Egg masses with approximately <100 eggs were excluded from the experiment because of possible abnormal hatchability. Five egg masses were inoculated into the bag or container contents used in the raw, silage, and control groups. To investigate the effect of states during and after silage preparation, including compaction and anaerobic condition, in the raw group (n = 5), blister-stage whole-crop corn was shredded using the cutting machine with a cutting length of 19 mm. Of the shredded corn, 100 g was inoculated with five FAW egg masses and sealed in a vacuum packaging plastic bag with vacuum. To evaluate the effect status after silage fermentation, including fermentation products and pH, in the silage group (n = 5), 100 g of blister-stage whole-crop corn silage, which was stored for approximately 1 y postpreparation, was inoculated with five egg masses and sealed using the method similar to that used in the raw group. In the control group (n = 5), artificial feed was inoculated with five egg masses and placed in plastic containers, allowing air ventilation. All bags and containers were incubated at 20°C for 3 days. This incubation period was designed because of the 2- to 3-day duration of the egg stage during warm summer months (Prasanna et al. 2018). Because no hatched eggs were found during the incubation period in any group, all bags and containers were opened after incubation. Subsequently, all egg masses were isolated from the bag and container contents. Individual egg masses were incubated with artificial feed at 20°C for 5 days. Then, egg hatchability was determined via microscopic counting of the number of hatched eggs.

3. Silage fermentation evaluation

To evaluate silage fermentation, pH measurement and organic acid analysis were performed. The obtained bag contents were extracted with 100 mL of reverse osmosis water per 25 g of contents overnight at 4°C and then filtered with a 5A quantitative filter paper (Advantec Toyo Kaisha, Tokyo, Japan). The pH of the extracts was measured using HI 2002-01 edge (Hanna Instruments, Smithfield, RI, USA). Organic acid concentrations were determined using the postlabeling method with bromothymol blue via high-performance liquid chromatography (HPLC) (Jikyuushiryou riyou kenkyuukai 2009). The extract was subjected to an ion-exchange resin (Amberlite IR120B(H)-HG, Organo, Tokyo, Japan) and centrifuged at 12,000 rpm at room temperature. The supernatant was filtered with a 0.45-μm cellulose acetate membrane filter unit (DISMIC-13CP 13CP45AN, Toyo Roshi Kaisha, Tokyo, Japan), and the filtrate was used for HPLC analysis. The LC-2000 Plus system (JASCO Corporation, Tokyo, Japan) was used for HPLC, and an ultraviolet–visible detector (UV-2070 Plus, JASCO Corporation) was used to detect 445-nm absorbance. The Shodex RSpak KC-811 (Showa Denko, Tokyo, Japan) and Shodex RSpak KC-G 8B (Showa Denko) systems were used as the column and precolumn, respectively.

4. Statistical analysis

All statistical analyses were performed using R 4.0.5 (R Core Team, Vienna, Austria).

In the vacuum experiment, between-group differences in larval viability, pH, and organic acid concentrations were analyzed using Kruskal–Wallis rank sum test. When significant effects ($P < 0.05$) were detected, pairwise Student's *t*-tests were performed while treating variances as unequal and correcting for

multiplicity using Hochberg's method.

In the CO_2 experiment, differences in larval viability were analyzed using a method similar to that used in the vacuum experiment. The effects of incubation time and enclosed gas on pH and organic acid concentrations were analyzed by two-way analysis of variance. When significant interactions ($P < 0.05$) were detected, multiple comparison test was performed using Tukey's honestly significant test.

In the egg hatchability experiment, egg hatchability was analyzed using a mixed-effects model with treatment as the fixed effect, each bag or container as the random effect, and variance differences among treatments using the lme function of the nlme package. When a significant treatment effect ($P < 0.05$) was detected, estimated marginal means (emmean, also known as "least squares means") were estimated using the emmeans function. All pairwise differences between treatments were tested with Tukey's multiplicity adjustment using the contrast function of the emmeans package version 1.6.1 (Lenth 2021). The pH and organic acid concentrations of the shredded corn or silage postincubation were analyzed using Welch's two-sample *t*-test by considering the variances of dependent variables as unequal.

Results

1. Larval survival experiment

(1) Vacuum experiment

After incubation, differences in material grass inoculated larval viability were examined between states of with or without vacuum during silage preparation. The dry matter (DM) content of the silage material corn leaves was 18.8%.

Figure 1 shows the effects of vacuum on the larval viability rate of FAWs. The viability rate in the unsealed group incubated under aerobic conditions for 14 days was 61.0 ± 5.4% (n = 5). After sealing and 24 h of incubation, no viable larvae were observed in the vacuumed group (n = 5), whereas the average larval viability rate was 84.0 ± 11.4% (n = 5) in the sealed group. After 14 days of incubation, no viable larvae were observed in the vacuumed or sealed group.

The pH and organic acid concentrations of bag content extracts are presented in Table 1. After 24 h of incubation, pH was higher and lactic and acetic acid concentrations were lower in the sealed group than in the vacuumed group. In addition, pH was found to be higher and lactic acid and acetic acid concentrations were found to be lower in the sealed group after 24 h of incubation than those in the sealed and vacuumed groups after 14 days of incubation. Although the sealed group after 14 days of incubation showed higher propionic acid concentrations than those of the sealed or vacuumed group after 24 h of incubation, these concentrations were <0.01% of the fresh matter. Butyric, isobutyric, valeric, and isovaleric acids were not detected from any bag content extracts.

(2) Carbon dioxide experiment

After 24 h or 14 days of incubation, the effect of enclosing air or CO_2 during silage preparation on material grass inoculated larval viability was investigated. The DM contents of the corn leaves and stems were 27.5% and 17.8%, respectively. The calculated DM content of the material grass was 22.6%.

Figure 2 shows the effects of enclosed CO_2 on the larval viability rate. After 24 h of incubation, no viable larvae were observed in the CO_2 group, whereas the average viability in the air group was 35.0 ± 16.6%. After 14 days of incubation, no viable larvae were observed in both the CO_2 and air groups, with an average viability rate of larvae fed in the control group of 10.0 ± 12.2%.

Table 2 shows the pH and organic acid concentrations of the bag content extracts during the CO_2 experiment. The pH was significantly lower in the CO_2 group than in the air group and after 14 days than after 24 h of incubation. No significant interaction was detected in terms of pH. Lactic and acetic acid concentrations were significantly higher after 14 days than after 24 h of incubation, whereas no significant difference was observed between the CO_2 and air groups. Propionic acid was undetected in almost all bags (data not shown), and butyric, isobutyric, valeric, and isovaleric acids were not detected in any bag.

2. Egg hatching experiment

Differences in egg hatchability after sealing with vacuum were examined between inoculation to raw forage corn and corn silage. The DM contents of fresh corn and corn silage enclosed with egg mass were 17.9% and 17.8%, respectively. Figure 3 shows the FAW egg hatchability of the egg hatching experiment. In the control group, the estimated egg hatchability was 65.8%, which was higher than that of the other two groups ($P < 0.01$). In the raw group, almost no eggs were hatched, and the estimated hatchability was 0.05%. In the silage group, although the estimated hatchability was 7.9%, considerable differences in hatchability were observed between egg masses, resulting in highest and lowest values of 75% and 0%, respectively. No significant differences in hatchability were noted between the raw and silage groups.

Table 3 shows the pH and organic acid concentrations of the bag content or enclosed silage in the silage group

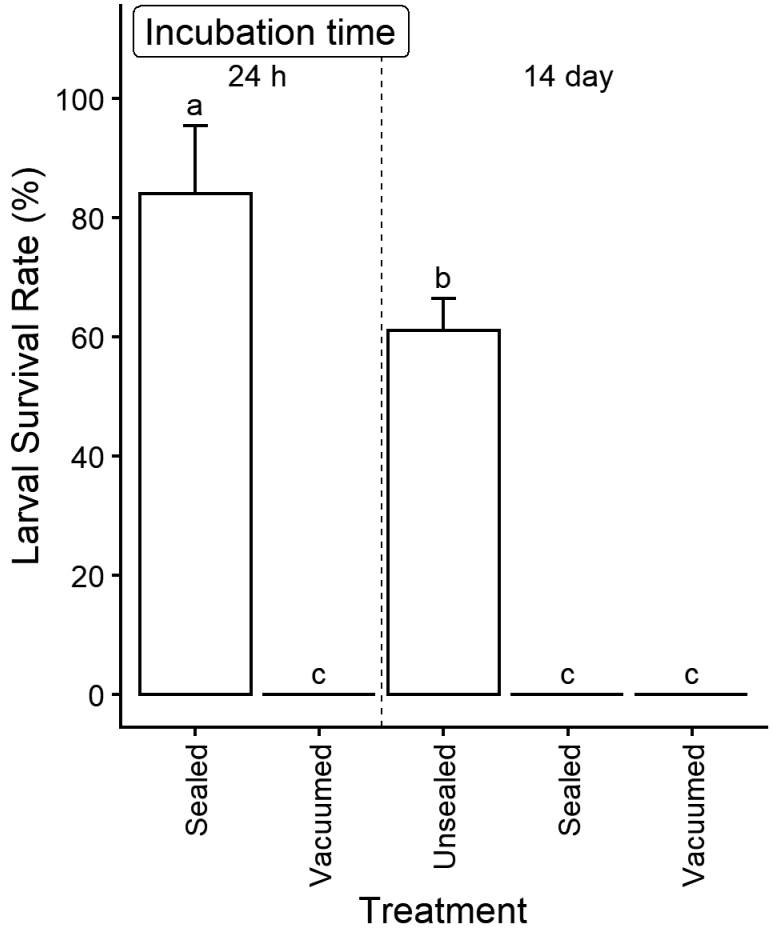

Fig. 1. Mean fall armyworm larval viability rate after 24 h or 14 days of incubation in a vacuum environment

Sealed group: larvae and shredded corn were sealed in a plastic bag without vacuum (n = 5 for 24 h and 14 days of incubation). Vacuumed group: larvae and shredded corn were sealed with vacuum (n = 5 for 24 h and 14 days of incubation). Unsealed group: larvae were fed under aerobic conditions (n = 5 for 14-day incubation period only). Error bars represent standard deviation. a, b, and c indicate statistically significant differences ($P < 0.05$).

Table 1. pH and organic acid concentration of bag content extracts in the vacuum experiment

Incubation time	24 h				14 days				*P*
Treatments	Sealed (n = 5)	Vacuumed (n = 5)	Unsealed (n = 5)	Sealed (n = 5)	Vacuumed (n = 5)				
pH	6.6 ± 0.0 a	5.5 ± 0.2 c	6.7 ± 0.1 a	6.0 ± 0.0 b	5.4 ± 0.4 bc				<0.01
Organic acid concentration (%, fresh matter)									
Lactic acid	0.000 ± 0.000 b	0.038 ± 0.024 ab	0.014 ± 0.008 ab	0.045 ± 0.017 ab	0.224 ± 0.121 a				<0.01
Acetic acid	0.001 ± 0.001 d	0.017 ± 0.006 c	0.099 ± 0.054 bcd	0.179 ± 0.033 ab	0.216 ± 0.032 a				<0.01
Propionic acid	ND b	ND b	0.003 ± 0.001 a	0.007 ± 0.003 ab	0.005 ± 0.005 ab				<0.01

Data are presented as mean ± standard deviation.

a, b, c: Letters indicate significant differences ($P < 0.05$) within rows.

ND: Not detected (<0.001%, fresh matter)

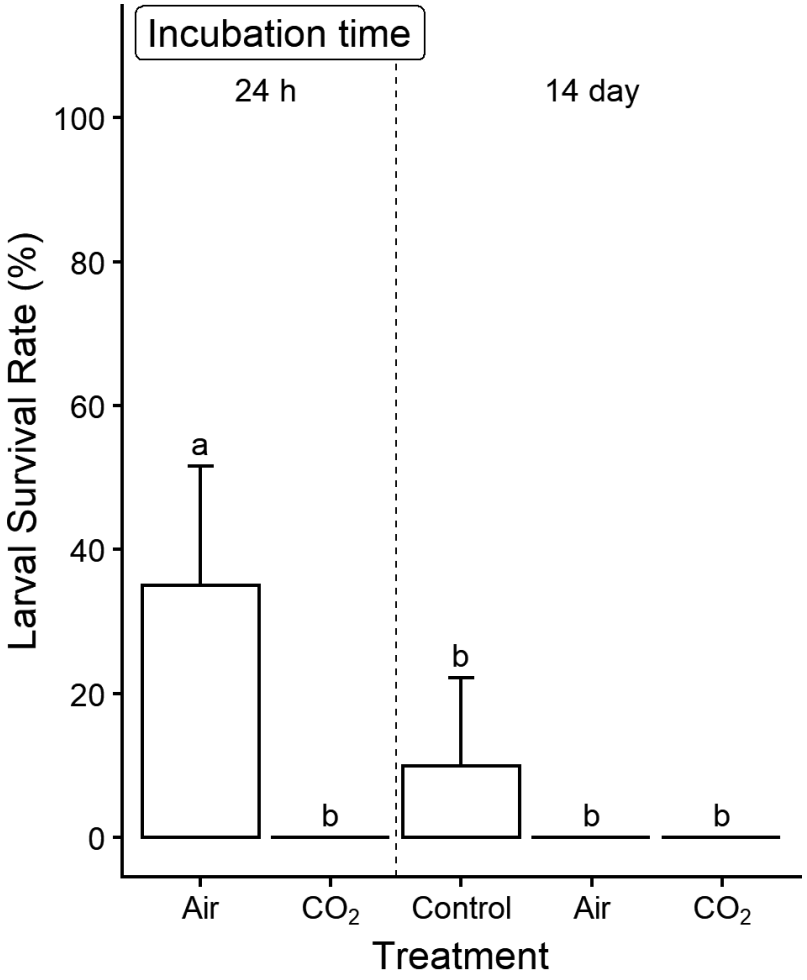

Fig. 2. Mean fall armyworm larval viability rate after 24 h or 14 days of incubation in a carbon dioxide environment

Air group: larvae and shredded corn were sealed in a plastic bag with ambient air (n = 5 for 24 h and 14 days of incubation). CO_2 group: larvae and shredded corn were sealed with carbon dioxide gas (n = 5 for 24 h and 14 days of incubation). Control group: larvae were fed under aerobic conditions (n = 5 for 14-day incubation period only). Error bars represent standard deviation. a and b indicate statistically significant differences ($P < 0.05$).

Table 2. pH and organic acid concentration of bag content extracts in the carbon dioxide experiment

Incubation time	24 h		14 days		*P*		
Treatment	Air (n = 5)	CO_2 (n = 5)	Air (n = 5)	CO_2 (n = 5)	Treatment	Incubation time	Interaction
pH	5.1 ± 0.1	4.9 ± 0.1	4.5 ± 0.1	4.2 ± 0.1	< 0.01	< 0.01	0.5
Organic acid concentration (%, fresh matter)							
Lactic acid	0.01 ± 0.01	0.02 ± 0.01	0.93 ± 0.14	1.10 ± 0.17	0.096	< 0.01	0.12
Acetic acid	ND	0.01 ± 0.00	0.23 ± 0.02	0.21 ± 0.01	0.13	< 0.01	0.098

Data are presented as mean ± standard deviation.
ND: Not detected (<0.01%, fresh matter)

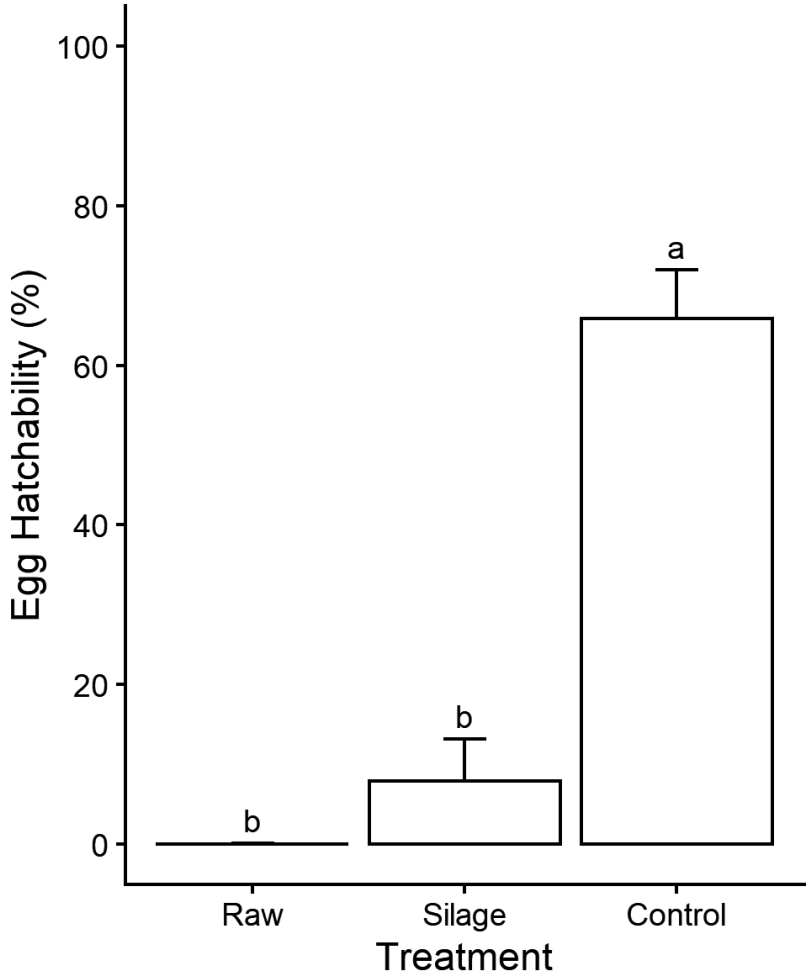

Fig. 3. Estimated marginal means of egg hatchability for fall armyworm egg masses incubated with shredded corn or corn silage sealed with vacuum

Raw group: egg masses were incubated with raw shredded corn in vacuum (n = 5). Silage group: egg masses were incubated with corn silage in vacuum (n = 5). Control group: egg masses were incubated under aerobic conditions (n = 5). Error bars represent standard error. a and b indicate statistically significant differences ($P < 0.05$).

Table 3. pH and organic acid concentration of the bag content and enclosed silage of silage group extracts in the egg hatchability experiment

Treatments	Raw (n = 5)	Silage (n = 5)	*P*	Enclosed silage (n = 1)
pH	4.1 ± 0.0	3.7 ± 0.0	< 0.01	3.7
Organic acid concentration (%, fresh matter)				
Lactic acid	1.35 ± 0.31	2.60 ± 0.16	< 0.01	2.30
Acetic acid	0.21 ± 0.06	2.46 ± 0.11	< 0.01	2.24
Propionic acid	0.00 ± 0.00	0.48 ± 0.02	< 0.01	0.44
Isobutyric acid	ND	0.01 ± 0.00	< 0.01	ND

Data are presented as estimated marginal mean ± standard error.
ND: Not detected (<0.01%, fresh matter)

extracts. The raw group showed higher pH and lower lactic acid and acetic acid concentrations than those of the silage group. Propionic and isobutyric acids were detected in the silage group but not in the raw group. The enclosed silage of the silage group showed lower lactic acid, acetic acid, and propionic acid concentrations than those in the silage group. Isobutyric acid was not detected in the enclosed silage.

Discussion

In this study, two experiments were performed to investigate factors that were affected after silage preparation, which in turn affected FAW larval viability under experimental conditions. In the vacuum experiment, no larvae survived in the vacuumed group after 24 h of incubation. The viability of the larvae in the vacuumed group was substantially lower than that of the larvae of the sealed group after 24 h of incubation. Further, the extracts of the vacuumed group showed lower pH than that of the sealed group after 24 h of incubation. Furthermore, after 24 h of incubation, lactic acid was detected at low concentrations in the extracts of the vacuumed group whereas it was undetectable in the sealed group, indicating that silage fermentation had started only in the vacuumed group after 24 h of incubation. After 14 days of incubation, no viable FAW larva was seen in the sealed group, similar to the observations in the vacuumed group after 24 h and 14 days of incubation. In addition, high pH and low concentrations of lactic acid were detected in the bag content extracts in the sealed group after 14 days of incubation, indicating the late initiation of silage fermentation in the sealed group compared with that in the vacuumed group. The results of the vacuum experiment indicated that any changes in the internal conditions of sealed bags after silage preparation substantially lowered the viability of FAW larvae in the vacuumed and sealed groups. In the vacuumed group, in addition to changes in the internal conditions of the bags, compression due to compaction also contributed to decreasing FAW larval viability. Thus, FAW larval viability decreased after 14 days of incubation with or without vacuum during silage preparation. However, the specific factors of the internal conditions affecting larval viability could not be identified based on the results of the vacuum experiment. In the CO_2 experiment, no viable larvae were observed in the CO_2 group, whereas 30% larvae were viable in the air group after 24 h of incubation. Furthermore, the pH and lactic acid concentration of the bag content extract were similar in both groups after 24 h of incubation, indicating almost no progress in silage fermentation. Thus, anoxic condition was found to be the main factor for the low larval viability in the CO_2 experiment.

In the air group, no viable larvae were detected after a 14-day incubation period, probably due to the anoxic condition created by aspiration of material grass. We concluded that compression and anaerobic conditions created by vacuuming and material grass aspiration were the major factors contributing to the decreased FAW larval viability in the vacuumed group after 24-h and 14-day incubation periods. Conversely, the anaerobic condition created by material grass aspiration was found to be the major factor for the low FAW larval viability after 14 days of incubation in the sealed and air groups of the vacuum and CO_2 experiments, respectively. The effects of pH or organic acid on FAW larval viability could not be assessed in the larval survival experiments because of higher pH and lower organic acid concentrations than those of usual well-fermented corn silage. However, regardless of the effect of pH or organic acids on FAW larval survivability, the anaerobic condition created by material grass aspiration is expected to reduce FAW larval survivability during favorable silage fermentation.

In the egg hatching experiment, the difference in effects was compared between the process after silage preparation (raw group) and the result of silage fermentation (silage group), and the effect of vacuuming was similar because both groups were similarly packaged with vacuum. The hatchability of the raw group was markedly lower than that of the control group after 3 days of incubation, indicating that changes accompanied with silage fermentation affected FAW egg hatchability. Lower pH and higher organic acid concentrations were observed in the silage group than in the raw group, whereas egg hatchability was higher in the silage group. These results indicated an insignificant impact of low pH and high organic acid concentrations on FAW egg hatchability. Although compression due to compaction might contribute to egg hatchability, the difference in egg hatchability between the raw and silage groups cannot be clearly explained by compression because both groups were similarly vacuumed when packaging. The results suggest that an anoxic condition is a major factor affecting FAW egg hatchability after silage preparation.

Our experiments were performed over a short period in a laboratory setting, which represents a limitation of our study. Particularly in larval survival experiments, several factors, such as temperature, cutting length, and the DM content of the silage material, differed from those of the usual corn silage preparation in fields. However, the results of our FAW larval survival experiments

showed the effectiveness of anoxic conditions in decreasing larval viability. In general silage preparation without vacuum, creating an anoxic condition inside the silo using material grass aspiration and maintaining the airtightness of the silo to facilitate anoxic conditions postpreparation are some of the necessary conditions for good silage fermentation. Thus, in actual production fields, decreased FAW larval viability in anoxic conditions is expected after silage preparation. For decreased FAW egg hatchability, silage preparation is expectedly effective based on the results of our egg hatchability experiment, especially the very low hatchability in the raw group. In this regard, silo airtightness for anoxic conditions in silo is probably an important factor to reduce FAW larval viability. In stored grain insect pests (*Tribolium castaneum*), the effect of anoxic condition on decreasing viability is lost by a 0.5-mm-diameter pinhole of plastic bag (Serata et al. 2003). Anaerobic conditions may be lost by a very small pinhole even in large-scale silo. Thus, maintenance of airtightness is very important.

Meanwhile, the effectiveness of the whole operation from forage corn harvesting to silage preparation against FAW outbreak remains undetermined. The harvesting operation using a harvester may spread FAW larvae to a certain large area, and then those larvae may spread by ballooning, a process facilitated by silk strand production and wind, to further nearby fields (Sokame et al. 2020, Zalucki et al. 2002). However, our results showed that FAW larval viability and egg hatchability rapidly and substantially decreased after silage preparation. Thus, there should be minimal concern regarding the spread of FAW from opened well-fermented silage.

We could not find previous studies regarding the effects of silage preparation on the viability of insect larvae or hatchability of eggs. As discussed, our results suggest that anoxic conditions after silage preparation decrease FAW larval viability. Given that oxygen is essential for the respiration of aerobic organisms, anoxic gas treatment is used to protect stored food commodities, agricultural products, and museum artifacts from several insect pests (Bell 2014, Maekawa & Elert 2003). Anoxic conditions after silage preparation and storage may eliminate several species of insect pest, both their larvae and eggs. Although the response to anoxic conditions has already been investigated in some insect species, the response in most of the feed crop pest insect species remains unknown. In the Lepidoptera *T. leucotreta*, larval exposure to anoxia for 12 h-36 h at 23°C decreased the pupation and emergence rates after their return to aerobic conditions (Boardman et al. 2016). In another Lepidoptera *Neptis rivularis*, hibernating larvae showed a 71% survival rate after 21 days of exposure to anoxic conditions, and this rate remained unchanged in aerobic conditions (Konvička et al. 2002). After 5 days of CO_2 exposure at 15°C, egg hatchability was further decreased to 1.7% in *Ephestia cautella* and 21.1% in *E. kuehniella* Zeller (Bell et al. 1980). Considering these results with our findings, anoxia tolerance in insect larvae and eggs appears to be highly variable across species. Thus, future studies should evaluate the viabilities of other insect pest species after silage preparation.

In conclusion, FAW early-instar larvae viability was quickly decreased by anoxic conditions after experimental-scale corn silage preparation. FAW egg hatchability also decreased after experimental-scale corn silage preparation. Our results suggest that airtightness is required to decrease FAW larval viability and egg hatchability after corn silage preparation.

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