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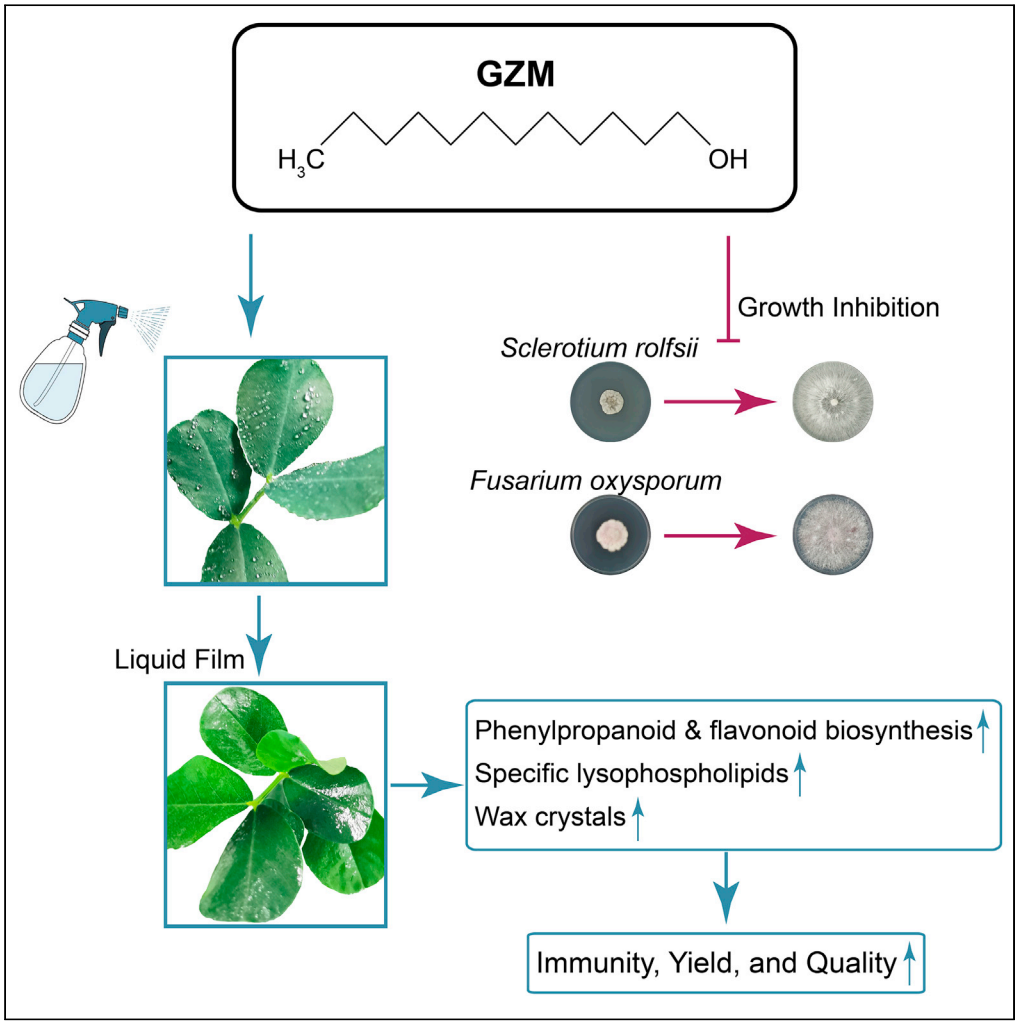
The plant protection preparation GZM improves crop immunity, yield, and quality



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Highlights

GZM provides more physiological protection than a physical protective film

GZM enhances phenylpropanoid, flavonoid, and wax biosynthesis in various plants

GZM and lauryl alcohol raise the contents of several specific lysophospholipids

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The plant protection preparation GZM improves crop immunity, yield, and quality

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SUMMARY

Lauryl alcohol, a natural compound found in plants and other organisms, is widely used to make surfactants, food, and pharmaceuticals. GZM, a plant protection preparation with lauryl alcohol as its major component is thought to establish a physical barrier on the plant surface, but its physiological functions are unknown. Here, we show that GZM improves the performance of peanut (*Arachis hypogaea*) plants in both the laboratory and the field. We demonstrate that the treatment with GZM or lauryl alcohol raises the contents of several specific lysophospholipids and induces the biosynthesis of phenylpropanoids, flavonoids, and wax in various plant species. In the field, GZM improves crop immunity, yield, and quality. In addition, GZM and lauryl alcohol can inhibit the growth of some pathogenic fungi. Our findings provide insights into the physiological and biological effects of GZM treatment on plants and show that GZM and lauryl alcohol are promising preparations in agricultural production.

INTRODUCTION

During their entire life cycle, plants are exposed to many stresses, including biotic and abiotic factors, which have a negative effect on their germination, growth, development, productivity, and postharvest preservation.[1–3] Indeed, these stresses contribute significantly to the loss of crop production worldwide with an average yield decline of 65%–85%.[4–6] Therefore, plants have acquired a series of effective mechanisms that can provide protection or become activated under stress conditions.[1]

In addition to the cell wall, synthetic physical barriers that coat the plant surface are critical for crops to resist biotic and abiotic stresses, such as drought, low temperature, ultraviolet (UV) damage, diseases, and pests.[7,8] Dozens of such physical protective agents have been developed and form a film on the plant surface, with good effects on yield and performance following their applications.[9–12] In the 1980s, a reagent under the tradename gao-zhi-mo in Chinese (GZM, meaning "high-quality lipid membrane") was developed and achieved good results for plant protection in the field, with a lower cost and less environmental damage than those in traditional plant protection preparations.[13] The main component of GZM is lauryl alcohol; 1-hexadecanol and Tween 80 are auxiliary components for emulsification and film formation.[13] Based on tests conducted by the Chemical Testing Institute of the Ministry of Agriculture of China, GZM is considered to be relatively innocuous, with no known toxic effects on the environment at the concentrations used in the field.[13] GZM is generally thought to act as a physical barrier to protect plants from pathogen invasion, as it forms a continuous liquid film or membrane on the plant surface when applied at a very low concentration.[13] However, action as a physical barrier cannot solely explain the efficacy of GZM. Despite the low cost of production and application of GZM, the lack of understanding of the specific mechanism behind the effectiveness of this chemical has hindered its popularization.

Lauryl alcohol (1-dodecanol), the main component of GZM, is a natural product found in female ginseng (*Angelica sinensis*), sacred lotus (*Nelumbo nucifera*), and other organisms. It is widely used to make surfactants and pharmaceuticals, as well as to enhance flavor in food.[14–16] However, the physiological effects of lauryl alcohol on plants are unknown.

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High-throughput "omics" technologies, such as transcriptomics and metabolomics have become powerful approaches to analyze genes, metabolites, and pathways related to the responsiveness of a plant to potential physiological intervention agents.[17–19]

In plants, the general phenylpropanoid biosynthetic pathway is one of the most important metabolic pathways related to biotic and abiotic stimuli.[20] Phenylpropanoid-based secondary products, like lignin and flavonoids, derived from the general phenylpropanoid biosynthetic pathway contribute substantially to the mechanical stability and robustness of plants against environmental damage, like drought or wounding,[21] and they have multiple roles in plant defense against pathogens and herbivores,[22–24] antioxidant activity,[25,26] protection against UV damage,[27–29] legume nodulation,[30,31] and control of auxin transport.[31] Plants that exhibit increased biosynthesis of phenylpropanoid-based secondary products under biotic and abiotic stresses usually acclimate better to adverse environments.[6,20] Therefore, the transcript levels of the key genes involved in the general phenylpropanoid biosynthetic pathway and concentrations of phenylpropanoid-based secondary products in plant tissue are ideal indicators of the extent of stress tolerance of various plants.[6]

The enzyme phenylalanine ammonia-lyase (PAL) catalyzes the formation of cinnamic acid from phenylalanine, which acts as the critical regulatory step between primary and secondary metabolism.[20,32] Numerous studies have shown the corresponding response of PAL activity to various biotic and abiotic stresses[32–35] Lysophosphatidylethanolamine (LPE)[36] is a biologically active lipid that can enhance PAL activity,[37] prime the plant immune system, promote basal resistance against hemibiotrophic pathogens,[38] regulate plant senescence and aging,[39–41] and induce plant growth.[40] Lysophosphatidylcholine (LPC), another biologically active lysophospholipid,[36] like LPE can also mediate a variety of cellular responses, including plant growth, development, systemic responses after wounding, and responses to stress and defense.[42–45]

Thus, activation of the general phenylpropanoid biosynthetic pathway and stimulation of specific plant metabolites may offer environmentally friendly and natural solutions[46,47] to reduce losses from crop production and improve crop performance through various approaches, including application of plant bio-stimulant products.

The main component of GZM is lauryl alcohol, which is a biosurfactant. Biosurfactants are considered to be less toxic and more eco-friendly than traditional chemical pesticides and use of green compounds to achieve sustainability in agriculture is a priority.[48,49] We show here, from work conducted on multiple crops grown in the laboratory and in field experiments that treating plants with GZM improves crop immunity, yield, and quality. Moreover, we demonstrate that the biosynthetic pathways for phenylpropanoids, flavonoids, and wax are activated upon GZM application and that several specific lysophospholipids accumulate. These results offer new clues as to the physiological effects of GZM and lauryl alcohol on plants and hold important theoretical significance for the promotion and application of GZM as a promising product in agriculture.

RESULTS

GZM improves peanut seed germination and seedling growth

Seed coating is a promising technique for the large-scale inoculation of crop seeds with adhesive agents, including GZM, to improve seed performance, alleviate biotic and abiotic stresses, and reduce production costs.[50–55] We investigated whether GZM deployed as a seed coating agent might improve the germination rate of seeds and early performance during the seedling stage. We coated peanut (*Arachis hypogaea*) seeds (cultivar Yuhua 9805) with different concentrations of GZM 18 months after seed harvest, a storage interval normally associated with poor germination, and scored germination rates and seedling growth under normal conditions in soil and under drought (simulated with 15% [w/v] polyethylene glycol [PEG] solution) or high-salt (provided by 1.5% [w/v] NaCl solution) conditions. Seeds treated with GZM showed greater germination potential and the resulting seedlings fared better than did controls, as evidenced by seedling height after 7 days of growth (Figures 1A–1C and Table S1). Furthermore, under drought and high-salt conditions, seedlings whose seeds were coated with GZM grew taller than control seedlings 3 days after germination (Figures 1D and 1E and Table S1). According to the dose effects observed at 7 days after germination, the seeds coated with higher concentrations of GZM showed greater germination potential and the seedlings fared better than those obtained from the seeds treated with lower concentrations of GZM (Figures 1D and 1E and Table S1). Lauryl alcohol, but not Tween 80, also improved seedling

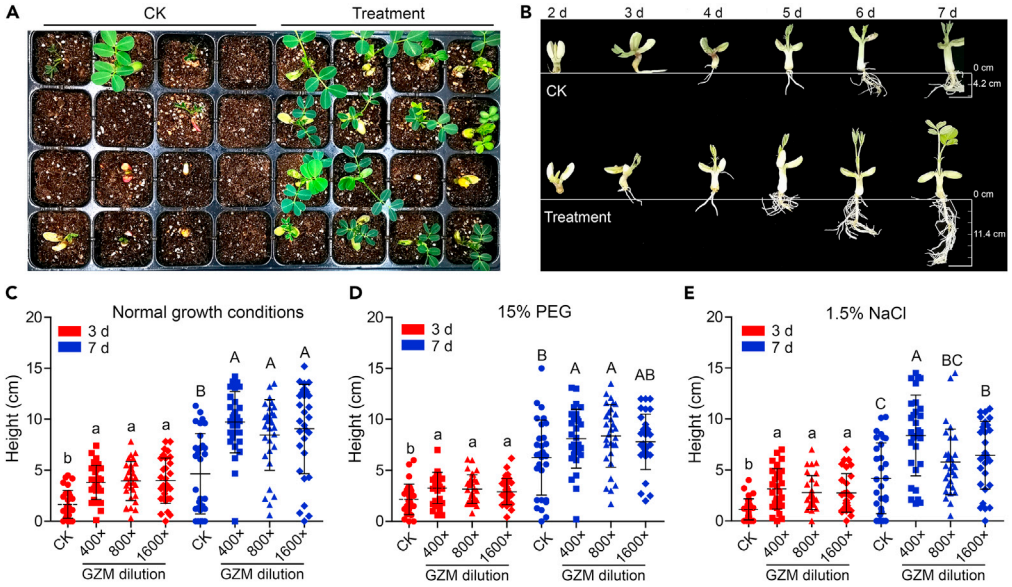

Figure 1. GZM improves seed germination and seedling growth of peanuts

(A) Peanut seeds (cultivar Yuhua 9805, 18 months after collection) were coated with a GZM stock solution (400x dilution) and then sown on soil. Seeds treated with sterile water served as the control (CK) groups. Photographs were taken 10 days later to record germination. Three biological replicates were conducted.

(B–E) Peanut seeds were coated with the indicated GZM dilutions (400x, 800x, and 1,600x in sterile water) or treated with sterile water and then sown on soil (B, C) or soaked in 15% (w/v) PEG2000 solution (D) or 1.5% (w/v) NaCl solution (E). Seedling growth was analyzed at the indicated time points. Data are represented as mean ± SEM (n = 30 germinated seeds). Individual seedlings are represented as separate symbols. Three biological replicates were conducted. Duncan's tests were performed after evaluating homoscedasticity data. Different letters indicate significant ($p < 0.05$) differences (one-way ANOVA). Source data are available in Table S1.

growth (Figure S1 and Table S1). These results demonstrate that GZM in general and lauryl alcohol in particular, improves peanut seed germination and seedling growth, even under stress conditions.

GZM improves performance of peanut and soybean in agricultural settings under stress conditions

To assess the potential usefulness of GZM in practical applications, we measured multiple parameters for peanut and soybean (*Glycine max*, cultivar Huachun No.1) plants grown in the field, where they suffered a severe drought during the growing period. Plants treated with GZM (four concentrations at different growth stages) accumulated more plant biomass and produced more seeds than did control plants (Figure 2). In addition, in-field GZM treatment diminished the occurrence of disease symptoms on the leaves of peanut plants and increased the number of root nodules on soybean plants (Figures 2A and 2D). Using peanut as a representative crop, we specifically measured a series of physiological and biochemical indicators. Peanut plants treated with GZM exhibited a marked increase in physiological indexes related to plant growth, such as plant height, number of tillers, diameter of straws, total chlorophyll contents, blade size, and percentage of fresh active root nodules (Figures 3A–3H and Table S2). GZM treatment also increased seed yield, fresh weight of pods, ratio of pods, and oil content of pods relative to control plants (Figures 3I–3K and Table S2). The numbers of mildewed pods (Figure S2 and Table S2) and rotten pods decreased after GZM treatment compared to controls (Figures 3L and 3M and Table S2). GZM treatment also decreased the incidence of disease spots on leaves (Figure 3N and Table S2) caused by *Cercospora* leaf spot disease.[56]

To explore the effects of GZM treatment on pest and disease prevention, we grew GZM-treated and control peanut plants under laboratory conditions and fed their leaves to third-stage tobacco cutworm (*Spodoptera litura*) (Fabricus) larvae, which constitute a serious threat to peanut plants, in the same insect breeding environment. We detected fewer instances of leaf consumption on the leaves of plants treated

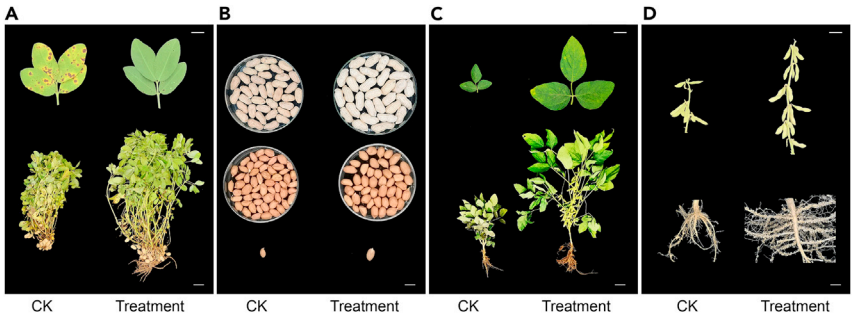

Figure 2. GZM improves performance of peanut and soybean during agricultural production

Peanut and soybean seeds were coated with GZM solution (400x dilution) as treatment group or sterile water as the control (CK) group and then sown in farmland. Leaves were then sprayed four times during the seedling stage, flowering stage, seed setting stage, and seed mature phase.

(A) Photographs of representative plants and leaves of peanut plants were taken 1 week before harvest. Scale bars, 1 cm (top), 5 cm (bottom).

(B) Photographs of peanut seeds were taken after harvest. Scale bar, 1 cm.

(C) Photographs of representative plants and leaves tissues of soybean were taken 1 week before harvest. Scale bars, 2 cm (top), 5 cm (bottom).

(D) Photographs of representative seeds and root tissues of soybean were taken 1 week before harvest. Scale bars, 2 cm (top), 1 cm (bottom). Three biological replicates were conducted.

with GZM than on those of control plants (Figure 3O and Table S2), suggesting that GZM treatment increased plant resistance against herbivores. As greater tolerance against pests is often associated with reactive oxygen species (ROS) contents, we measured the activity of several redox-related enzymes, as well as proline and malondialdehyde (MDA) contents. Proline (PRO) is associated with increased tolerance against pathogens and abiotic stress, while MDA is a marker of lipid peroxidation due to the accumulation of ROS. Compared to the control group, GZM-treated fresh leaves showed greater accumulation of PRO and higher activity levels for superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) but lower MDA content (Figures 3P–3T and Table S2). Taken together, these results indicate that treatment with GZM improves multiple related morphological and physio-biochemical traits of field crops in agricultural field settings.

We also assessed whether GZM treatment affected phytohormone levels in the leaves of peanut plants grown in the field. Many plant hormones involved in biotic and abiotic stress responses, such as salicylic acid, gibberellin A19, jasmonic acid, and abscisic acid, were more abundant in GZM-treated plants than in control plants, whereas cytokinin and auxin, which are related to growth regulation, were less abundant in GZM-treated plants than in control plants (Figure 4A and Table S3). As the soil microbiome can influence plant fitness, we purified DNA from the rhizosphere soil and analyzed the composition of the rhizosphere microbiome associated with field-grown peanut plants. Gammaproteobacteria, Alphaproteobacteria, and Bacteroidia, all known to play important roles in disease resistance and growth promotion,[57,58] were enriched in the rhizosphere of GZM-treated plants relative to controls (Figures 4B and S3). These results demonstrate that GZM treatment affects phytohormone contents and the composition of rhizosphere microorganisms in plants grown under field production conditions.

GZM upregulates phenylpropanoid, flavonoid, and wax biosynthesis in various plants

In previous studies, GZM application formed a protective film on the plant surface.[13] We noticed that spraying peanut, soybean, and Arabidopsis (*Arabidopsis thaliana*) leaves with a mixture of lauryl alcohol and Tween 80 (1.3 mM lauryl alcohol, 23.3 μM Tween 80) was effective in covering the leaf surface, even that of hairy leaves (Figure S4). However, during test experiments, we discovered that spraying the leaves of Arabidopsis and soybean plants with high concentrations of GZM (five times higher than the typically used concentration) results in a cell death–like phenotype within 10 h of treatment (Figure S5). This observation suggested that GZM might not only form a physical barrier on leaves but also quickly change the physiological properties of treated plants. We performed transcriptome and metabolome profiling of leaves from 4-week-old peanut plants treated with GZM diluted 1,000 times (corresponding to concentrations of 1.3 mM lauryl alcohol, 123.7 μM 1-hexadecanol, and 23.3 μM Tween 80). We identified 529

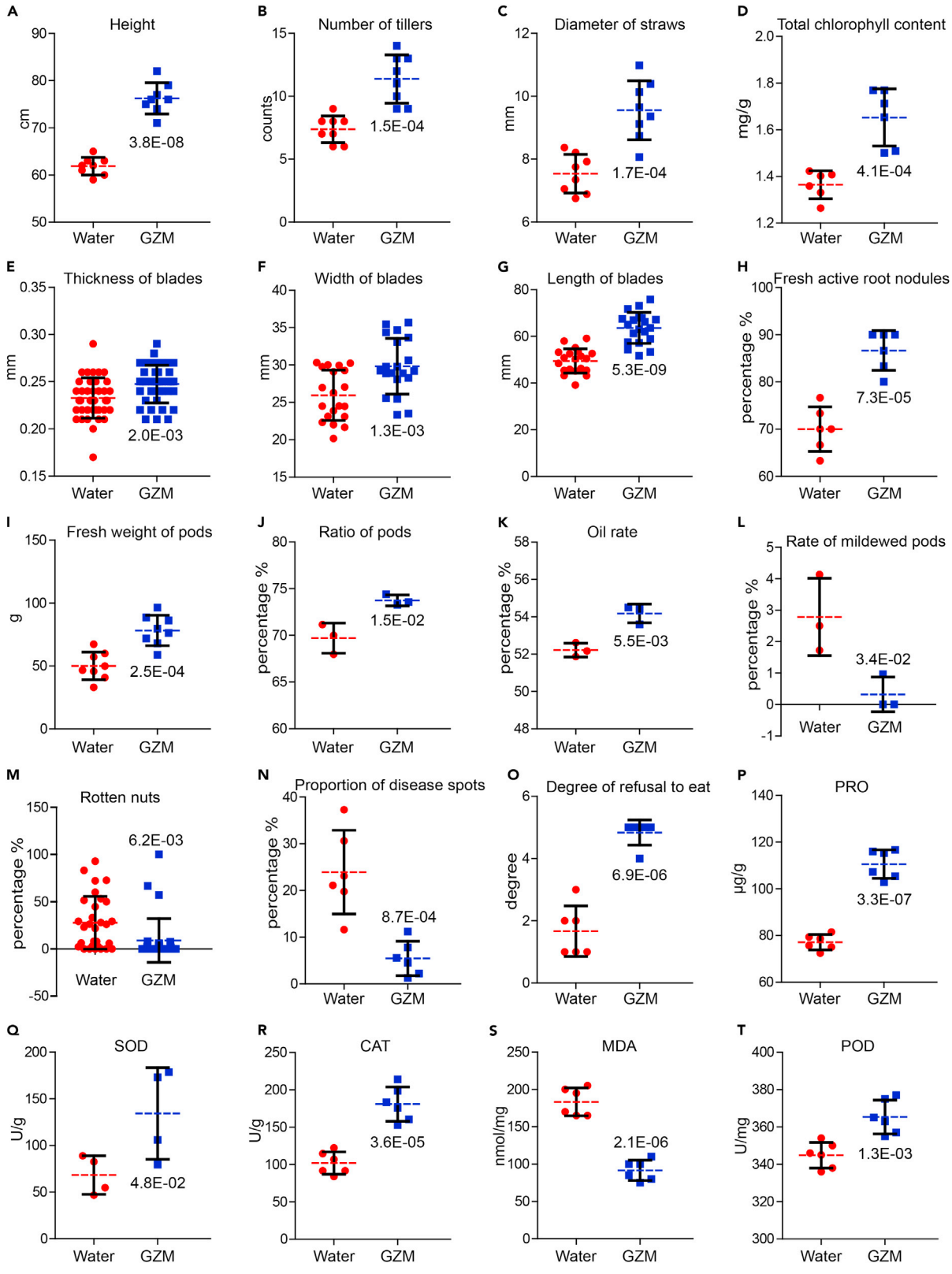
A
Height
cm
3.8E-08
Water
GZM
B
Number of tillers
counts
1.5E-04
C
Diameter of straws
mm
1.7E-04
D
Total chlorophyll content
mg/g
4.1E-04
E
Thickness of blades
mm
2.0E-03
F
Width of blades
mm
1.3E-03
G
Length of blades
mm
5.3E-09
H
Fresh active root nodules
percentage %
7.3E-05
I
Fresh weight of pods
g
2.5E-04
J
Ratio of pods
percentage %
1.5E-02
K
Oil rate
percentage %
5.5E-03
L
Rate of mildewed pods
percentage %
3.4E-02
M
Rotten nuts
percentage %
6.2E-03
N
Proportion of disease spots
percentage %
8.7E-04
O
Degree of refusal to eat
degree
6.9E-06
P
PRO
μg/g
3.3E-07
Q
SOD
U/g
4.8E-02
R
CAT
U/g
3.6E-05
S
MDA
nmol/mg
2.1E-06
T
POD
U/mg
1.3E-03

Figure 3. Morphological, physiological, and biochemical traits of peanuts with or without GZM treatment

(A–C) Morphological indexes of peanut plants grown in the field (1 week before harvest) treated with GZM or with sterile water (Water) as control (n = 8 samples/group).

(D–H) Physiological and morphological indices of peanut leaves: total chlorophyll contents (D), thickness (E), width (F), and length of blades (G), and percentage of fresh active root nodules (H). E, n = 40 samples/group; F and G, n = 20 samples/group; H, n = 6 samples/group; three biological replicates were conducted.

(I–M) Indices for yield and seed storage: fresh weight of pods (I), ratio of pods (J), oil rate (K), rate of mildewed pods (L), and rotten pods (M). I, n = 8 samples/group; J–L, n = 3 samples/group; M, n = 30 samples/group. Three biological replicates were conducted.

(N) Proportion of disease spots on leaves due to *Cercospora* leaf spot disease. n = 6 samples/group. Each sample consisted of six leaves of six different plants. Three biological replicates were conducted.

(O) Peanut plants (Yuhua 9805) were grown under laboratory conditions; the leaves of GZM-treated and control plants were offered to third-stage larvae of *Spodoptera litura* (Fabricus). The extent of herbivory activity was scored after 12 h of feeding. n = 6 samples/group. Each sample consisted of six leaves from different plants. Three biological replicates were conducted.

(P–T) PRO (P) and MDA (S) contents and SOD (Q), CAT (R), and POD (T) activity in GZM-treated and control fresh leaves. P, R–T, n = 6 samples/group; Q, n = 4 samples/group. Each sample consisted of four leaves of four different plants. Three biological replicates were conducted. Data are represented as mean ± SEM. Duncan's tests were performed after evaluating homoscedasticity data. The p values compared to the control group are provided (Student's *t* test). Source data are available in Table S2.

differentially expressed genes (DEGs) between plants treated with GZM and control plants (Figure 5A and Table S4). Among them, 428 genes were upregulated in GZM-treated plants, while the remaining 101 genes were downregulated (Table S4). A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that these DEGs were significantly enriched in the pathways of plant–pathogen interaction, mitogen-activated protein kinase (MAPK) signaling, isoflavonoid biosynthesis, and alpha-linolenic acid metabolism (Figure 5B and Table S5). We also identified 62 metabolites with greater abundance and 53 metabolites with lower abundance in GZM-treated peanut leaves than in controls (Figure 5C and Table S6). KEGG analysis of these differentially abundant metabolites also revealed an enrichment for the isoflavonoid biosynthesis pathway in GZM-treated leaves compared to controls (Figure 5D and Table S7). Most of the 62 more abundant metabolites were flavonoids and phenolic acids (Table S6). Of these, the top 10 more abundant metabolites belonged to lignans and coumarins, amino acids and derivatives, flavonoids, phenolic acids, nucleotides and derivatives, and lipids (Figure 5E and Table S6). Together, the transcriptome and metabolome results indicate the enrichment of the isoflavonoid biosynthesis pathway in leaves treated with GZM compared to controls (Figure 5F). In addition, we observed the transcriptional upregulation of genes encoding various enzymes involved in isoflavonoid biosynthesis (Figure S6, Tables S4 and S6). These results suggest that GZM treatment induces isoflavonoid biosynthesis in peanut plants.

To assess whether this effect is conserved across other members of the Leguminosae family, we measured metabolite contents in kudzu (*Pueraria lobata* [Willd.] Ohwi), with high flavonoid contents accumulating in its dried roots (named Radix *Puerariae*).[59] Among 141 metabolites with significant differences in abundance, 135 were more abundant in GZM-treated samples than in controls (Figure 6A and Table S8). Consistent with the results in peanuts, isoflavonoid biosynthesis was the most highly enriched pathway in *P. lobata* (Figure 6B and Table S9), with isoflavonoids, anthocyanins, flavones, and flavonols accumulating in GZM-treated plants compared to controls (Figures S7–S9 and Table S8). The effects of GZM treatment on the isoflavonoid biosynthesis pathway also extended outside the Leguminosae, as leaves of *Nicotiana benthamiana*, from the Solanaceae family, displayed greater contents of flavonoids when treated with GZM than when treated with water (Figure S10, Tables S10 and S11). We expanded our transcriptome analysis to soybean (Leguminosae family), Arabidopsis (Cruciferae family), wheat (*Triticum aestivum* L.; Poaceae family), and tomato (*Solanum lycopersicum*; Solanaceae family), as well as the epidermis of navel orange (*Citrus sinensis* Osb. Var. *brasiliensis* Tanaka) fruits (Rutaceae family), which accumulate variable levels of flavonoids without GZM treatment. Genes participating in the phenylpropanoid and flavonoid biosynthesis pathways in these plants were significantly upregulated in samples treated with GZM compared to controls (Figures S12–S28 and Tables S12–S21), suggesting that GZM has a widespread and shared effect on plants.

GZM is used as a spray preparation and comes into direct contact with the plant surface. Considering that the wax layer in plants plays an important protective role against biotic and abiotic stresses,[8,29] we used scanning electron microscopy to observe the effect of GZM treatment on the wax crystals on the surface of peanut leaves. We noticed more wax crystals on the surface of leaves treated with GZM three times during leaf development than on the surface of control leaves (Figure 7A). We also observed the significant upregulation of key genes in the cutin and wax biosynthesis pathways in GZM-treated tissues compared

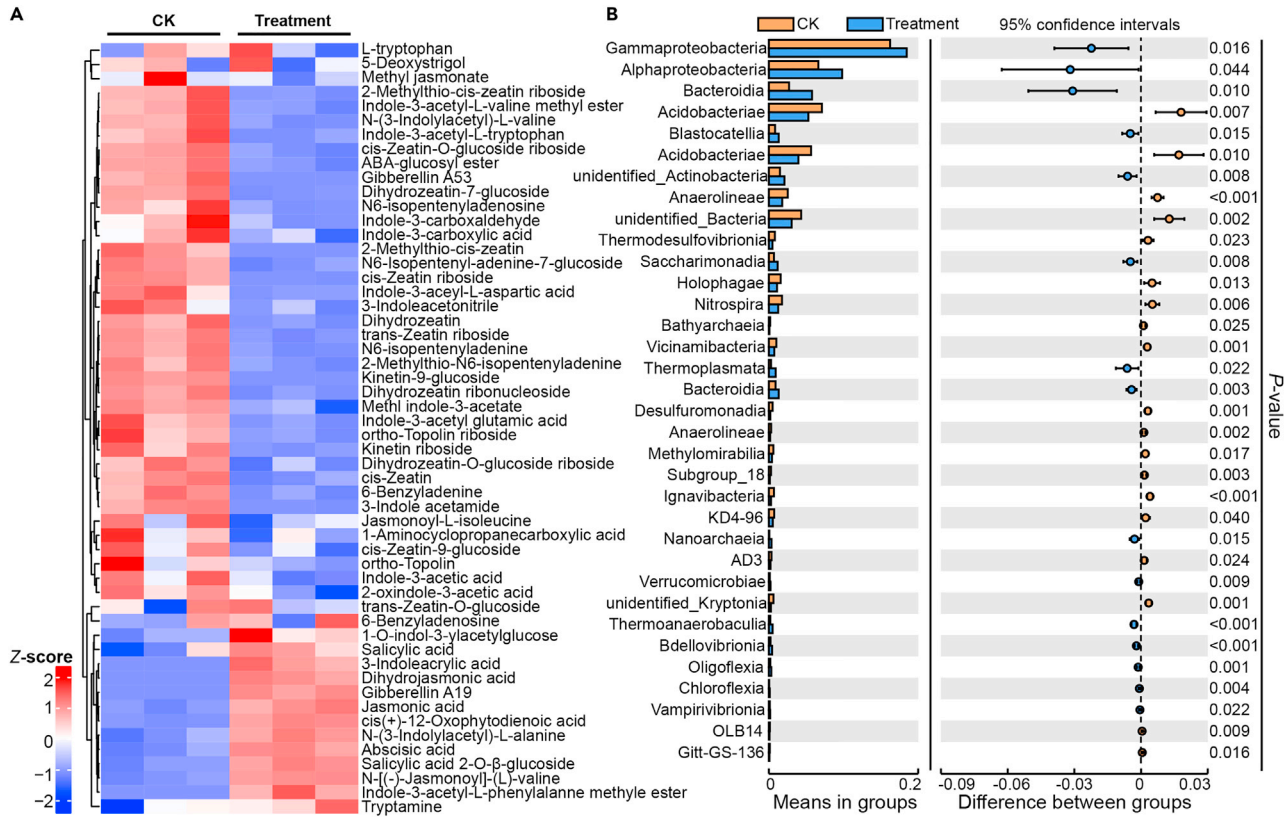

Figure 4. Plant hormone contents and composition of rhizosphere microorganisms

(A) Heatmap representation of all determined phytohormone contents after clustering analysis in the treatment and control (CK) groups. Peanut plants were grown and treated with GZM or sterile water as aforementioned, and the phytohormone contents in leaves were determined.

(B) Bacterial species exhibiting a significant difference in abundance in the rhizosphere of plants treated with GZM or with sterile water as control. Each bar represents the mean value of species abundance within each group, with the associated 95% confidence interval, normalized between groups, shown on the right. The center of the circle represents the difference of the within-group means, and its color indicates in which group the species is more abundant. Six biological replicates were conducted. Data are represented as mean ± SEM. p-values of intergroup significance tests (Duncan's test) are shown to the far right.

to controls (Figure 7B and Table S4). To assess whether this effect is conserved across different crops, we analyzed the transcriptome data of crops treated with GZM and detected the same molecular signature in all treated plants, indicating that the physiological role of GZM is conserved in different crops (Figure 7B, Tables S4, S12, S16, S18, and S20).

Taken together, these results demonstrate that GZM treatment can regulate phenylpropanoid, flavonoid, and wax biosynthesis in a conserved manner across different plant species.

GZM and lauryl alcohol induce the accumulation of lysophospholipids in various plant species

One of the top 10 more abundant metabolites in peanut leaves treated with GZM did not belong to either flavonoids or phenolic acids: the lipid lysophosphatidylcholine (LysoPC; 12:0) (Figures 5E and 8A). LysoPC and lysophosphatidylethanolamine (LysoPE) are lysophospholipids, important signaling molecules in response to biotic and abiotic stresses[42–44] that delay plant senescence and aging[40,60,61] and promote plant growth.[40] To determine whether LysoPC or other lipids accumulate in other plants, we looked for this compound in metabolome data obtained for GZM-treated peanut leaves, the fruit epidermis of navel oranges grown in the field, and tomato leaves grown in the laboratory. GZM treatment led to increased abundance of LysoPC (20:4) in peanut leaves, LysoPE (18:2) in navel orange fruit epidermis, and LysoPC (12:0) in tomato leaves (Figures 8B–8D and Tables S22, S23, and S24).

To determine the individual contributions of the main components of GZM to the accumulation of these metabolites, we treated leaves from various plant species with either lauryl alcohol or Tween 80 and carried

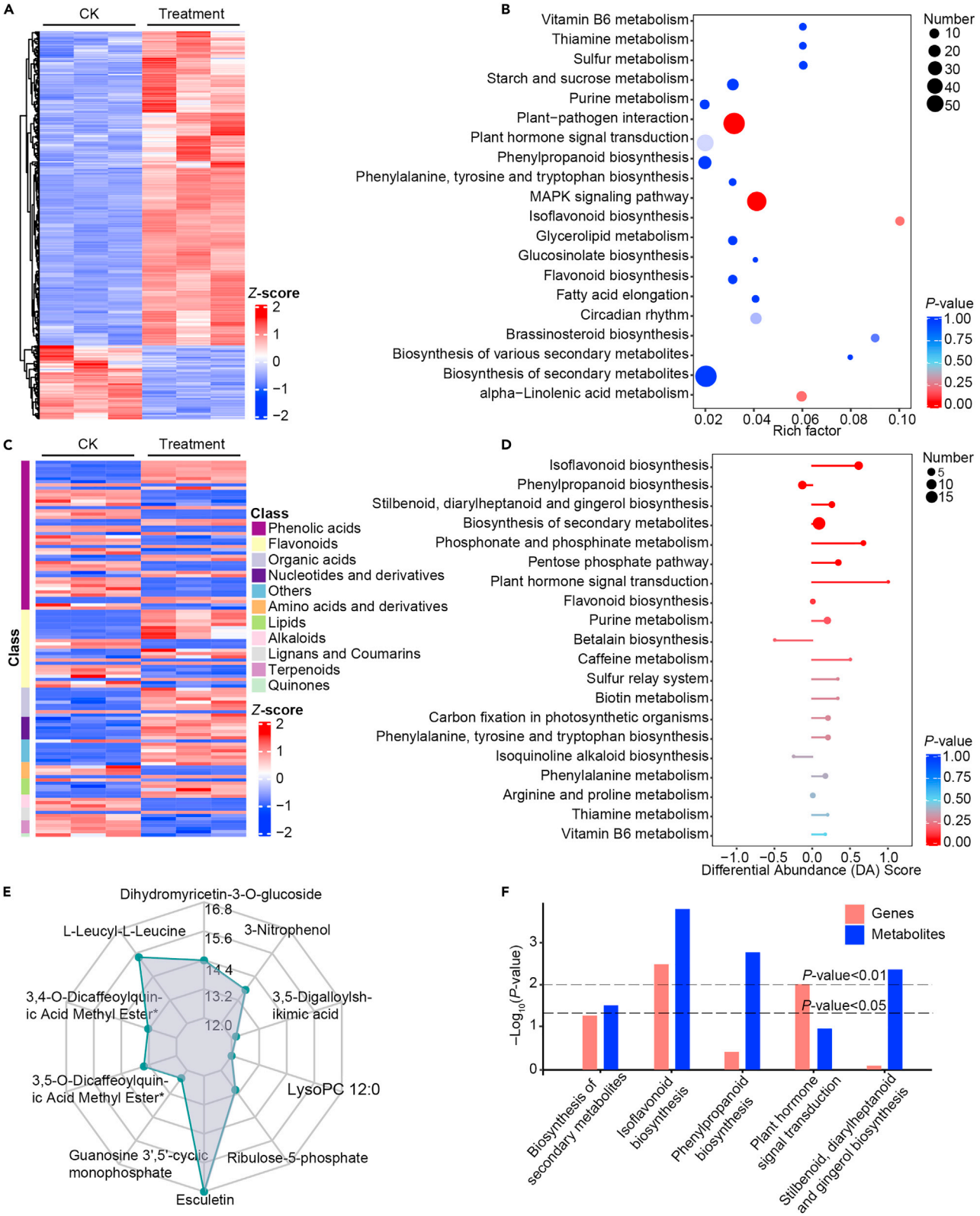
A
CK
Treatment
Z-score
2
1
0
−1
−2
B
Vitamin B6 metabolism
Thiamine metabolism
Sulfur metabolism
Starch and sucrose metabolism
Purine metabolism
Plant−pathogen interaction
Plant hormone signal transduction
Phenylpropanoid biosynthesis
Phenylalanine, tyrosine and tryptophan biosynthesis
MAPK signaling pathway
Isoflavonoid biosynthesis
Glycerolipid metabolism
Glucosinolate biosynthesis
Flavonoid biosynthesis
Fatty acid elongation
Circadian rhythm
Brassinosteroid biosynthesis
Biosynthesis of various secondary metabolites
Biosynthesis of secondary metabolites
alpha−Linolenic acid metabolism
0.02
0.04
0.06
0.08
0.10
Rich factor
Number
10
20
30
40
50
P-value
1.00
0.75
0.50
0.25
0.00
C
CK
Treatment
Class
Class
Phenolic acids
Flavonoids
Organic acids
Nucleotides and derivatives
Others
Amino acids and derivatives
Lipids
Alkaloids
Lignans and Coumarins
Terpenoids
Quinones
Z-score
2
1
0
−1
−2
D
Isoflavonoid biosynthesis
Phenylpropanoid biosynthesis
Stilbenoid, diarylheptanoid and gingerol biosynthesis
Biosynthesis of secondary metabolites
Phosphonate and phosphinate metabolism
Pentose phosphate pathway
Plant hormone signal transduction
Flavonoid biosynthesis
Purine metabolism
Betalain biosynthesis
Caffeine metabolism
Sulfur relay system
Biotin metabolism
Carbon fixation in photosynthetic organisms
Phenylalanine, tyrosine and tryptophan biosynthesis
Isoquinoline alkaloid biosynthesis
Phenylalanine metabolism
Arginine and proline metabolism
Thiamine metabolism
Vitamin B6 metabolism
−1.0
−0.5
0.0
0.5
1.0
Differential Abundance (DA) Score
Number
5
10
15
P-value
1.00
0.75
0.50
0.25
0.00
E
Dihydromyricetin-3-O-glucoside
16.8
15.6
14.4
13.2
12.0
3-Nitrophenol
3,5-Digalloylsh-
ikimic acid
LysoPC 12:0
Ribulose-5-phosphate
Esculetin
Guanosine 3',5'-cyclic
monophosphate
3,5-O-Dicaffeoylquin-
ic Acid Methyl Ester*
3,4-O-Dicaffeoylquin-
ic Acid Methyl Ester*
L-Leucyl-L-Leucine
F
Genes
Metabolites
P-value<0.01
P-value<0.05
−Log10(P-value)
0
1
2
3
Biosynthesis of
secondary metabolites
Isoflavonoid
biosynthesis
Phenylpropanoid
biosynthesis
Plant hormone
signal transduction
Stilbenoid, diarylheptanoid
and gingerol biosynthesis

Figure 5. GZM induces the isoflavonoid biosynthesis pathway in peanut plants

Peanut plants were grown under laboratory conditions for 4 weeks before their leaves were fully sprayed with atomized GZM solution, and then sprayed again 48 h later. Leaf samples were collected 72 h after the first spraying for transcriptome and metabolome analysis.

(A) Heatmap representation of all determined DEGs in the indicated samples after clustering analysis. The three biological replicates per condition are shown. CK, control group; treatment, GZM-treated group.

(B) KEGG pathway enrichment analysis of the DEGs. The top 20 most enriched pathways are shown.

(C) Heatmap representation of all determined differentially abundant metabolites in the indicated samples after clustering analysis.

(D) KEGG pathway enrichment analysis of the differentially abundant metabolites. The top 20 most enriched pathways are shown. The x axis represents the differential abundance (DA) score. A score of 1 indicates that the identified metabolites within a given pathway are more abundant, whereas a score of −1 indicates that the identified metabolites within a pathway are less abundant after treatment with GZM. The length of the line represents the absolute value of the DA score.

(E) Radar map of the differentially abundant metabolites. The top 10 most differentially abundant metabolites are shown on a Log_2(fold-change) scale.

(F) Degree of enrichment of pathways with both differentially abundant metabolites (blue) and DEGs (red). The y axis represents the p-values for enrichment, shown as $-Log_{10}$(p-value). n = 3 samples/group. Each sample consisted of six leaves from different plants. Three biological replicates were conducted.

out metabolome analysis of treated and control samples. Several lipids accumulated upon treatment with lauryl alcohol compared to treatment with Tween 80: LysoPC (12:0) and LysoPE (16:3) in peanut, LysoPC (12:0) in Arabidopsis, LysoPE (16:1, 2n isomer) and LysoPC (12:0) in rice (*Oryza sativa*), and LysoPC (12:0) and LysoPC (20:5) in wheat and *N. benthamiana* leaves (Figures 8E–8I and Tables S25–S29). Similarly, LysoPC (12:0), LysoPE (14:0, 2n isomer), and LysoPE (14:0) accumulated in tomato leaves treated with lauryl alcohol relative to control leaves (Figure 8J and Table S30). These results indicate that GZM and its main component lauryl alcohol can induce the accumulation of specific lysophospholipids in various plants.

GZM improves crop performance

We next assessed whether GZM treatment improves plant growth and postharvest preservation performance of crops and seeds. The diameter of rice straws from plants grown in the field and treated with GZM was 22.9% greater than that of controls; we observed no significant difference in plant height between the groups (Figure S29 and Table S31). Likewise, GZM treatment of tobacco (*Nicotiana tabacum*) increased growth rate, growth potential, leaf size, straw thickness, and root biomass (Figures S10A–S10D). In three independent field tests, GZM treatment improved tobacco yield by 20.3% relative to controls (Figure S10E). GZM treatment also affected fruit production. The fruit epidermis of Tribute Citru (*Citrus sinensis*) treated with GZM was smoother than that of controls (Figure 9A). Furthermore, the storage period of GZM-treated fruits at room temperature was prolonged compared to that of control fruits (Figures 9B and 9C). The occurrence of physiological premature drop of fruits and sunburned fruits markedly decreased following GZM treatment (Figures 9D–9F and Table S32), as did the extent of damage caused by the citrus leafminer *Phyllocnistis citrella* (Figure 9F and Table S32). We obtained similar results for Japanese plum (*Prunus salicina*) production: GZM treatment decreased the occurrence of physiological premature drop of fruits and the incidence of fruit cracking (Figures 9G and 9H and Table S32). Taken together, these results demonstrate that GZM has a good application value in production practices (Table S33).

GZM and lauryl alcohol can inhibit the growth of pathogenic fungi

Does GZM directly block the growth of pathogenic microbes rather than (or in addition to) providing a physical barrier against pathogens? We established that GZM and lauryl alcohol does inhibit growth of the pathogenic fungi *Fusarium oxysporum* f. sp. *cubense* and *Sclerotium rolfsii* in a dose-dependent manner (Table S34). Other components of GZM, such as 1-hexadecanol and Tween 80, had no effect (Figure 10). These results suggest that GZM and its main component lauryl alcohol can inhibit the growth of some pathogenic fungi.

DISCUSSION

GZM is believed to form a protective coat over leaves, providing a physical barrier against pathogens.[13] We confirmed here that spraying plant leaves with a mixture of lauryl alcohol and Tween 80 covered the leaf surface, even that of hairy leaves (Figure S4). This physical effect makes GZM a possible mixing agent with other pesticides to increase their plant surface adsorption and action. We determined that GZM directly induced strong physiological and physio-biochemical responses in plants. These results not only reveal the physiological mechanism of action of GZM but also suggest that other reagents, used in field production or during development to protect plants by forming a physical film, might also have practical applications in inducing plant physiological and physio-biochemical responses.

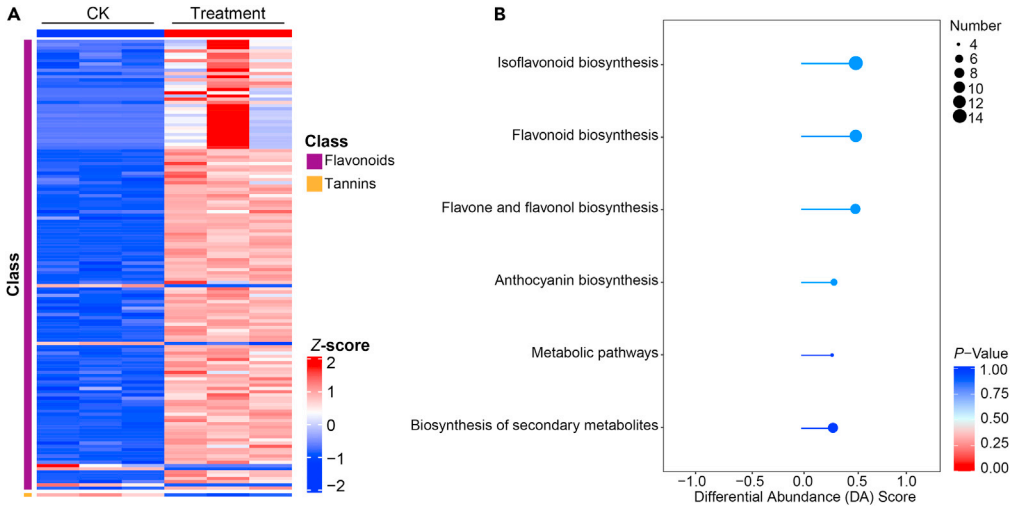

Figure 6. GZM induces the flavonoid biosynthesis pathway in kudzu (*Pueraria lobata* [Willd.] Ohwi)

Kudzu plants in the field treated with GZM or sterile water; samples were collected 1 week before harvest for metabolome analysis.

(A) Heatmap representation of all determined differentially abundant metabolites after clustering analysis in each replicate from the treated and control groups.

(B) KEGG pathway enrichment analysis of the differentially abundant metabolites. The top six most enriched pathways are shown. The x axis represents the DA score. n = 3 samples/group. Data are represented as mean ± SEM. Each sample consisted of three pieces of tissue from different plants. Three biological replicates were conducted.

Another practical reason for GZM use is its affinity toward the leaf surface, which increases the absorption and diffusion of traditional pesticides. We also established in this study that leaves treated with GZM showed a lower incidence of disease spots (Figure 3N and Table S2) caused by *Cercospora* leaf spot disease, as well as diminished damage caused by the citrus leafminer *P. citrella* (Figure 9F and Table S32). We cannot currently distinguish between a direct effect of GZM as an insect repellent or its effect on plant immunity, underscoring the need for further research. However, at the application level, these results showed that GZM is a promising preparation for boosting the affinity of agents for the plant surface during mixed application and might also serve as a carrier in the development of mixed green agents in the future.

In general, plants must balance their needs for growth with the demand for resources dedicated toward their immunity.[62,63] However, biological agents or plant endophytes can promote plant growth and even improve yield and product quality while also inducing immunity.[64–67] These reports suggest that the trade-off between defensive phenotypes and growth may be constrained by the allocation of limiting resources, while under stress conditions, the same trade-offs may become mutually supportive.

The phenylpropanoid and flavonoid biosynthesis pathways play critical roles in plant stress responses.[20,21] For instance, the induction of phenylpropanoid biosynthesis leads to an increase in lignin contents and raises plant resistance against diseases and pests.[20,21] Flavonoids are important plant metabolites that impart greater tolerance to UV damage and drought stress,[6,27–29,68] which may explain why straw diameter, chlorophyll contents, plant growth, and yield increased under stress conditions, while the incidence of plant diseases decreased (Figure 2). In addition, we showed that LysoPC and LysoPE were among the metabolites displaying the largest increase in contents in GZM-treated plants (Figure 8, Tables S22–S29, and S33). The transcriptome data showed that in some crops, such as peanuts, wheat, and navel orange, the transcript levels of phospholipase genes involved in the biosynthesis of LPE (Lysophosphatidylethanolamine) and LPC (Lysophosphatidylcholine)[36,42] were significantly upregulated in tissues treated with lauryl alcohol (Table S35). These results may provide an explanation for the increase in LPC and LPE contents in these species, but determining why the related genes are not all induced in all crops will require further research. LPE and LPC exist only in relatively small amounts in natural organisms.[36,42] They have a fatty acid group, and the results of metabolomics indicated that the composition of this group is similar to that of lauryl alcohol or *n*-hexadecanol, suggesting that the main components of GZM may act as precursors of LPE and LPC and participate in the physiological biosynthesis pathway in plants after external

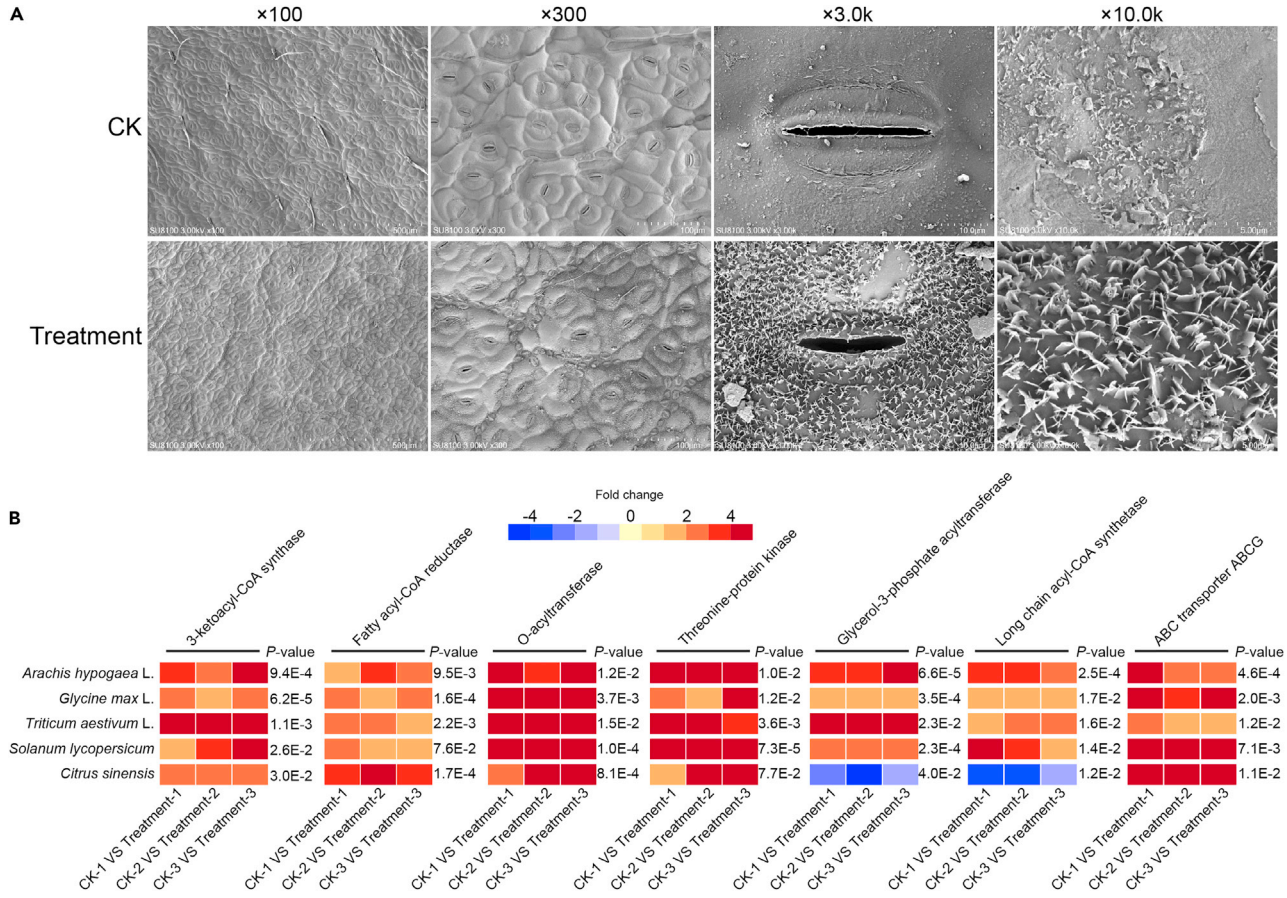

Figure 7. Effects of GZM on plant epicuticular wax

(A) Scanning electron microscopy image of peanut leaves. Peanut leaves were fully sprayed with atomized GZM solution (treatment group) or sterile water (control group) at 3 weeks, 5 weeks, and 7 weeks after seed germination. Leaf samples were collected 1 week after the third spraying for imaging. The symbols of ×100, ×300, ×3.0k, and ×10k indicate the magnification. n = 6 samples/group. Three biological replicates were conducted.

(B) Heatmap representation of transcript levels of key genes involved in wax biosynthesis in GZM-treated plants and control plants. Three biological replicates were conducted. Each biological replicate from the treatment group was compared to the control group. Data are represented as mean ± SEM. The p-values compared to the control group are provided (Student's *t* test).

application. Further research, for example isotope labeling and tracing, is required to analyze differences in the types of accumulated lysophosphatides across different crops and to understand how lauryl alcohol increases the contents of specific lysophosphatides. LysoPC and LysoPE are lysophospholipids that promote plant growth,[40] delay plant senescence and aging,[39,40] and induce stress and defense responses.[38,44,45] The consistent accumulation of lysophospholipids upon GZM treatment suggests they are key factors in the effects of GZM treatment on plant physiology. Lysophospholipids can raise activity of phenylalanine ammonia-lyase (PAL), the rate-limiting enzyme in phenylpropanoid, and flavonoid biosynthesis.[37] This observation may explain the increased abundance of both lysophospholipid and flavonoid in various plants upon treatment with GZM. In this study, we showed that *PAL1* transcript levels were induced in peanuts, Arabidopsis, wheat, tomato, and navel orange upon GZM treatment, whereas we detected no changes in *PAL1* expression in soybean, kudzu, rice, tobacco, or *N. benthamiana*. However, the aim of the transcriptome sequencing was to specifically examine *PAL1* transcript levels; in the future, targeted exploration of changes in the transcript levels of other *PAL* paralogs in each crop will potentially indicate whether the level of PAL in cells is altered by GZM treatment.

In studying the rhizosphere microbiome of peanut plants, we determined that the abundance of Gammaproteobacteria, Alphaproteobacteria, and Bacteroidia increased significantly in the GZM-treated group relative to controls (Figure 4). Gammaproteobacteria are important in disease suppressiveness,[57] while

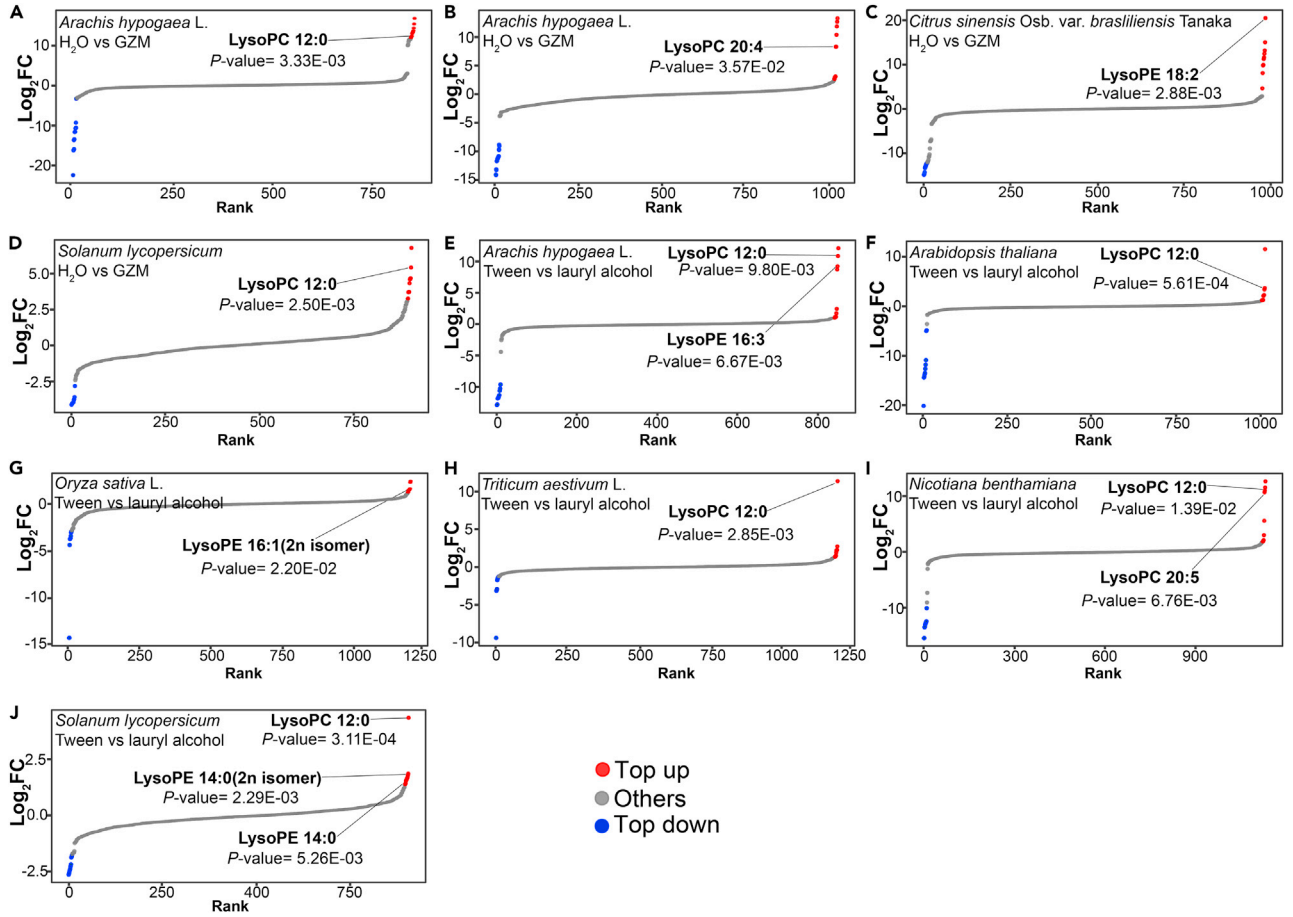

Figure 8. Dynamic distribution maps of differentially abundant metabolites

Fold-change (FC) values of metabolite contents between the different treatment groups, arranged in increasing order.

(A–D) Metabolite contents in plant tissues treated with 1,000× diluted GZM (corresponding to 1.3 mM lauryl alcohol, 123.7 μM 1-hexadecanol, and 23.3 μM Tween 80) compared to those of the groups treated with sterile water.

(E–J) Metabolite contents in plant tissues treated with lauryl alcohol (corresponding to 1.3 mM lauryl alcohol and 23.3 μM Tween 80) compared to those of the groups treated with Tween 80 (23.3 μM). The y axis shows Log_2(FC). Each point represents a substance; blue, top 10 downregulated compounds; red, top 10 upregulated compounds. n = 3 samples/group. Each sample consisted of six pieces of tissue from six different plants. Three biological replicates were conducted. Data are represented as mean ± SEM. The p-values compared to the CK group are provided (Student's *t* test). Source data are available in Tables S22–S29.

Bacteroidia population diversity can increase with the addition of sawdust in the soil.[58] It is possible that GZM altered the rhizosphere microbiome after leaching into the soil during spraying. Alternatively, GZM may induce physiological changes in plants that in turn alter the contents and/or types of root exudates, which would also affect the complement of rhizosphere microorganisms. The fact that we counted more effective nodules on the roots of Leguminous plants treated with GZM relative to control plants (Figures 2B and 3H) might support the second hypothesis.

We showed here that GZM and lauryl alcohol had almost the same inhibitory effect on the growth of *F. oxysporum* f. sp. *cubense*, and GZM was more effective than lauryl alcohol at preventing the growth of *S. rolfsii* (Figure 10 and Table S34). These results suggest that the small amount of 1-hexadecanol in GZM might contribute to the inhibitory effect of GZM on some specific microorganisms. However, 1-hexadecanol alone did not inhibit the growth of *S. rolfsii*, suggesting that it might have an auxiliary role during physical film-forming or that lauryl alcohol and 1-hexadecanol have synergistic effects.

In this study, we showed that GZM and lauryl alcohol deployed as a seed coating agent on peanut seeds improved seed germination rate and early performance, even under stress conditions, during the seedling

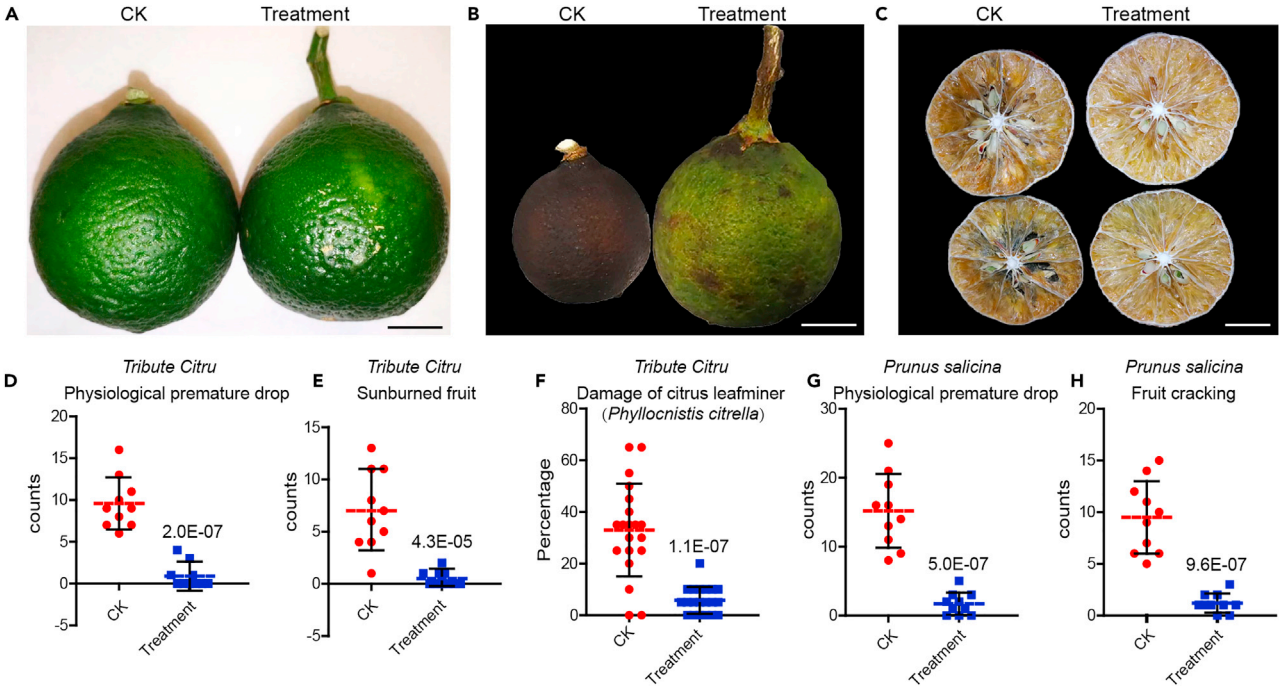

Figure 9. GZM treatment increases fruit production

(A–C) Representative photographs of *Tribute Citru* fruits from the treatment or control (CK) group at 1 day (A), 28 days (B), and 35 days (C) postharvest, followed by storage at room temperature. Scale bars, 2 cm.

(D and E) Incidence of the physiological premature drop of fruits (D) and sunburned fruits (E) in *Tribute Citru* production. n = 10 samples/group.

(F) Percentage of leaves damaged by citrus leafminer (*Phyllocnistis citrella*). n = 20 samples/group.

(G and H) Incidence of the physiological premature drop of fruits (G) and fruit cracking (H) during *Prunus salicina* production. n = 10 samples/group. Data are represented as mean ± SEM. The p-values compared to the CK group are provided (Duncan's test). Source data are available in Table S32.

stage. A recent study revealed that mitochondrial GPAT (glycerol-3-phosphate acyltransferase)-derived LPA controls auxin-dependent embryonic and postembryonic development.[69] Considering that GZM and its main component lauryl alcohol induce the accumulation of lysophospholipids in various plant species, and in light of the phytohormone contents of GZM-treated plants, we observed the accumulation of some plant hormones, including 3-indolebutyric acid, which can promote seed germination, stress tolerance, and root growth.[70–72] Therefore, we speculate that the effect of GZM and lauryl alcohol on seed germination and early plant performance may be due to the accumulation of specific lysophospholipids that lead to changes in the content of related phytohormones.

In practical applications, the recommended dilution of the GZM stock solution is 1,000 times (corresponding to 1.3 mM lauryl alcohol, 123.7 μM 1-hexadecanol, and 23.3 μM Tween 80). In our test experiments, we sprayed Arabidopsis and soybean leaves with a concentration of GZM solution that was five times higher than this recommended concentration for practical implementations (corresponding to 6.5 mM lauryl alcohol, 618.5 μM 1-hexadecanol, and 116.5 μM Tween 80), but observed a phenotype similar to cell death within 10 h (Figure S5). This phenomenon may be due to severe physiological and biochemical reactions upon treatment with GZM. Biosurfactants can damage plant cells at high doses,[48,49] and so this result might also suggest that GZM can act as a surfactant.

In addition to the effects of GZM on plant pathogens, leaves treated with GZM appeared to be less palatable to herbivores than control leaves (Figure 3O and Table S2), which was reflected in the contents of metabolites and phytohormones in treated leaves. Lauryl alcohol is one of the metabolites in the entomopathogenic fungus *Conidiobolus coronatus* that can affect the fatty acid composition and cellular immunity of the greater wax moth (*Galleria mellonella*) and blue blow flies (*Calliphora vicina*), suggesting that lauryl alcohol may play an important role during *C. coronatus* infection.[73] These observations raise the possibility that GZM might directly affect pest physiology. However, this hypothesis is not consistent with our

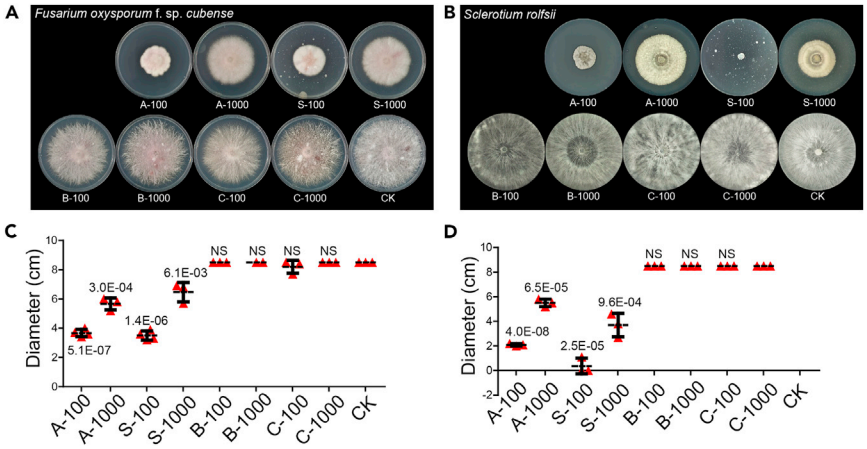

Figure 10. GZM and lauryl alcohol inhibit the growth of pathogenic fungi

(A and B) One piece (5-mm diameter) of agar medium covered with *Fusarium oxysporum* f. sp. *cubense* or *Sclerotium rolfsii* was placed in the center of each plate. S, medium containing GZM; A, medium containing lauryl alcohol; B, medium containing 1-hexadecanol; C, medium containing Tween 80. The numbers 100 and 1000 indicate the dilution of the corresponding compound. For control (CK) plates, an equivalent volume of sterile water was added.

(C and D) Mean diameter of the mycelial covers of *F. oxysporum* (C) and *S. rolfsii* (D) shown in (A, B). Three biological replicates were conducted. Data are represented as mean ± SEM. The p-values compared to the control groups are indicated (Duncan's test). Source data are available in Table S34.

observations in the field, where we failed to detect any effect for GZM on predatory insects or insect pollination, suggesting that lauryl alcohol may influence insect food intake instead.

In this study, we reported that GZM treatment induces the accumulation of several phytohormones, such as gibberellin (Figure 4A), and antioxidant enzymes (Figures 3Q, 3R and 3T), which are important for plant defenses against biotic and abiotic stresses.[74–78]

Limitations of the study

The most direct mechanism of the effect of GZM on plant physiology has not been clarified. Whether GZM is recognized by plant receptors or is directly involved in specific physiological metabolic pathways in treated plants will need further exploration.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106819.

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AUTHOR CONTRIBUTIONS

G.H.Y. conceived and supervised the project, and Y.H.S., G.H.Y., X.M.P., and D.X.C. designed the experiments. Y.H.S. performed most of the experiments. D.X.C., D.Q., J.L.C., Y.T.S., X.Y.Z., Z.M., J.Z., L.N.X., Z.Y.D., P.C., X.M.P., and G.H.Y. performed some of the experiments. Y.H.S. analyzed data and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Fusarium oxysporum f. sp. *cubense*	Sun et al.[65]	N/A
Sclerotium rolfsii	ATCC	ATCC 15004
Biological samples		
Spodoptera litura	Tiwari et al.[79]	N/A
Chemicals, peptides, and recombinant proteins		
GZM stock solution	Zhuhai Runnong Technology	N/A
Lauryl Alcohol	Sigma-Aldrich	W261718
Tween 80	Sigma-Aldrich	P8074
Critical commercial assays		
RNA Extraction Trizol Reagent	Thermo Fisher Scientific	15596026 and 15596018
RNA Integrity Assay Kit	Univ-bio	CB19714965
QiaQuick PCR Extraction Kit	Qiagen	28104
Proline Content Detection Kit	Nanjing Jiancheng Bioengineering Institute	A107-1-1
MDA Content Detection Kit	Nanjing Jiancheng Bioengineering Institute	A003-1
SOD Activity Determination Kit	Nanjing Jiancheng Bioengineering Institute	A001-3
POD Activity Determination Kit	Nanjing Jiancheng Bioengineering Institute	A084-3
Catalase (CAT) Activity Assay Kit	Sangon Biotech	D799598
Fixative solution	Servicebio	G1102-100 ML
Deposited data		
Raw and analyzed data (*Arabidopsis thaliana*)	This paper	PRJNA827264
Raw and analyzed data (*Citrus sinensis*)	This paper	PRJNA827341
Raw and analyzed data (*Arachis hypogaea* L.)	This paper	PRJNA827338
Raw and analyzed data (*Glycine max* L.)	This paper	PRJNA827520
Raw and analyzed data (*Solanum lycopersicum*)	This paper	PRJNA827281
Raw and analyzed data (*Triticum aestivum* L.)	This paper	PRJNA827926
Raw and analyzed data (rhizosphere microbiome)	This paper	PRJNA827859
Experimental models: Organisms/strains		
Arabidopsis thaliana	ABRC	Columbia-0
Arachis hypogaea L. (Yuhua 9805)	Henan Academy of Agricultural Sciences, China	N/A
Oryza sativa L. (Huanghua Zhan)	Guangdong Academy of Agricultural Sciences, China	N/A
Triticum aestivum L. (Yunong 211)	Henan Academy of Agricultural Sciences, China	N/A
Nicotiana benthamiana	NCBI	Taxonomy ID 4100
Solanum lycopersicum (Money Maker)	NCBI	Taxonomy ID 4081
Glycine max L. (Huachun No.1)	South China Agricultural University, China	N/A
Software and algorithms		
ImageJ	N/A	https://imagej.nih.gov/ij/
KOBAS	http://kobas.cbi.pku.edu.cn/kobas3/genelist/	http://kobas.cbi.pku.edu.cn/kobas3/genelist/

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Adobe Illustrator 2020	N/A	https://www.adobe.com/cn/products/illustrator.html
Microsoft Excel	Microsoft, Richmond USA	https://www.microsoft.com
GraphPad Prism	GraphPad Prism	RRID:SCR_002798
Uparse algorithm	Uparse v7.0.1001	http://www.drive5.com/uparse/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Guohui Yu (ygh76411@zhku.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- The transcriptome sequencing data mentioned in this study have been deposited at the SRA database of NCBI under the following accession numbers: PRJNA827264 (*A. thaliana*), PRJNA827341 (*Citrus sinensis*), PRJNA827338 (*A. hypogaea* L.), PRJNA827520 (*G. max* L.), PRJNA827281 (*S. lycopersicum*), and PRJNA827926 (*T. aestivum* L.). The raw data for composition of the rhizosphere microbiome associated with field-grown peanut plants have been deposited under the accession number PRJNA827859.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plant and microbe cultivation

Plants of *A. hypogaea* L. (Yuhua 9805), *O. sativa* L. (Huanghua Zhan), *T. aestivum* L. (Yunong 211), *N. benthamiana*, *S. lycopersicum* (Money Maker), and *G. max* L. (Huachun No.1) were grown (26 ± 2°C with a 16-h light/8-h dark photoperiod and 70–80% relative humidity) in the greenhouse. *A. thaliana* plants (accession Columbia-0 [Col-0]) were grown under different conditions (21 ± 2°C with a 14-h light/10-h dark photoperiod and 70% relative humidity) in an artificial climate box.

F. oxysporum f. sp. *cubense* (FOC009) and *S. rolfsii* (ZHKSR) were cultured on PDA medium at 30°C.

METHOD DETAILS

Growth inhibition of microorganisms

For *F. oxysporum* f. sp. *cubense* and *S. rolfsii*, one piece (5 mm in diameter) of agar plate covered with a fresh fungal mass was cut with a hole puncher and placed in the center of a PDA (Potato Dextrose Agar) plate (with different concentrations of reagents or an equivalent volume of sterile water). Plates were incubated at 30°C until the mycelium of the control group filled the Petri plate; the diameter of the mycelial cover was then measured in each treatment group and representative plates were photographed.

Seed, plant, and microorganism treatment with GZM and components

Stock solutions of GZM or its constituent reagents were used at the same concentrations; lauryl alcohol (1.3 M), 1-hexadecanol (123.7 mM), and Tween 80 (23.3 mM). Sterile water was used as the solvent for the stock solutions and for dilution. A series of diluted GZM stock solutions were used in this study. For 400× dilution of GZM, the final concentrations of the constituent reagents were 3.3 mM lauryl alcohol, 309.3 μM 1-hexadecanol, and 58.3 μM Tween 80. For 800× dilution of GZM, the final concentrations of the constituent reagents were 1.6 mM lauryl alcohol, 154.7 μM 1-hexadecanol, and 29.1 μM Tween 80. For 1,000× dilution of GZM, the final concentrations of the constituent reagents were 1.3 mM lauryl alcohol,

123.7 μM 1-hexadecanol, and 23.3 μM Tween 80. For 1,600× dilution of GZM, the final concentrations of the constituent reagents were 815.0 μM lauryl alcohol, 77.3 μM 1-hexadecanol, and 14.5 μM Tween 80.

For peanut seeds treated under laboratory conditions, seeds (n = 390) (cultivar Yuhua 9805, 18 months after collection) were soaked in a series of diluted GZM stock solutions (400x, 800x or 1,600x dilutions of stock solutions with sterile water) or 800x dilutions of lauryl alcohol stock solution for 1 min, surface-dried, and then sown on soil under normal growth conditions, or seeds were half soaked in 15% (w/v) PEG2000 solution or 1.5% (w/v) NaCl solution for drought and high-salt pressure growth conditions. Seeds soaked in sterile water or Tween 80 (29.1 μM) served as the control (CK) groups. Seedling growth was analyzed on days 3, 5 and 7 after seed germination. Root lengths of seedlings or plants were measured. In actual production practice, peanut and soybean seeds were coated with GZM solution (400x dilution) as treatment group or soaked in sterile water as the control (CK) group before being sown in farmland. Three biological replicates were conducted each time.

For the treatment of leaves under laboratory conditions, the leaves of 4- to 5-week-old plants (n = 18) were fully sprayed with 1,000x diluted GZM (corresponding to 1.3 mM lauryl alcohol, 123.7 μM 1-hexadecanol, and 23.3 μM Tween 80) or lauryl alcohol (corresponding to 1.3 mM lauryl alcohol and 23.3 μM Tween 80) by manual atomizing spray. Leaves sprayed with sterile water or Tween 80 (23.3 μM) served as the control (CK) groups. Leaves were sprayed again 48 h later. Six leaves with similar status per plant were cut 72 h after the first spraying and quickly frozen in liquid nitrogen for 5 min and then transferred to a dry ice environment for storage until use.

For the growth inhibition tests of microorganisms (*F. oxysporum* f. sp. *cubense* and *S. rolfsii*), different concentrations of reagents or an equivalent volume of sterile water were mixed with the agar medium before pouring the plates. During the test using the Oxford cup method, 0.1 mL diluted solution or an equivalent volume of sterile water was added to the corresponding cup.

For the treatment of GZM on annual crops during field production, seeds were coated with GZM solution (400× dilution, corresponding to 3.3 mM lauryl alcohol, 309.3 μM 1-hexadecanol, and 58.3 μM Tween 80) as treatment group or sterile water as the control (CK) group before being sown; the leaves of established plants were then sprayed with 1,000x diluted GZM (corresponding to 1.3 mM lauryl alcohol, 123.7 μM 1-hexadecanol, and 23.3 μM Tween 80) four times at the seedling stage, flowering stage, seed setting stage, and seed mature phase, with a vapor-pressure type knapsack sprayer. For fruit production in the field, the same concentration of GZM solution was sprayed four times on leaves and fruits during the new bud formation stage, flowering stage, fruit setting stage, and fruit mature phase. Plants were sprayed with sterile water as the control (CK) group. Plants (n = 18) in different groups were randomly selected, and plant tissues and soil samples were collected 1 week before harvest (1 week after the fourth spraying) in field production, quickly frozen in liquid nitrogen for 5 min, and then transferred to a dry ice environment for storage until use. Field experiments of peanut (*A. hypogaea* L.), soybean (*G. max* L.), and navel orange (*Citrus sinensis* Osb. var. *brasiliensis* Tanaka) were performed in Zhangshi Town, Shaoguan City, Guangdong Province, China. Field production experiments for *Citrus* fruits were conducted in Tangtang Town, Guangzhou City, Guangdong Province, China. Experiments with Japanese plum (*P. salicina*) were performed in Dungang Town, Shaoguan City, Guangdong Province, China. Field experiments with tobacco (K326) and rice were performed in Mashi Town, Shaoguan City, Guangdong Province, China.

Transcriptomics, widely targeted metabolomics, phytohormones, and rhizosphere microbiome analyses

Samples were collected and immediately frozen in liquid nitrogen for 5 min and then transferred to a dry ice environment for storage until the determination of all omics data and phytohormone contents. The processing and testing of the samples were completed by MetWare (Wuhan, China) (http://www.metware.cn/).

For RNA extraction, library construction, and sequencing, Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) was used for total RNA extraction from all samples. RNA integrity was assessed using an RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). mRNA was isolated with oligo(dT) beads, then fragmented and reverse-transcribed into first-strand cDNA with random primers. The second strand of cDNAs was produced using DNA polymerase I, then purified with a QiaQuick PCR extraction kit (Qiagen, Hilden, Germany). The products were selected by

electrophoresis and PCR-amplified with Phusion High-Fidelity DNA polymerase, Index (X) Primer, and Universal PCR primers. The PCR products were purified using AMPure XP beads and clustered with a cBot Cluster Generation System, according to the manufacturer's instructions. Libraries were sequenced on an Illumina Hiseq platform, as paired-end reads of 125–150 bp. The raw data were filtered using fastp v 0.19.3. HISAT v2.1.0 was used to construct the demultiplex index and map the clean reads to the reference genome. The alignment and FPKM of each gene were calculated using featureCounts v1.6.2.

For sample preparation and extraction of widely targeted metabolomics, vacuum freeze-drying was used; the samples were placed in a lyophilizer (Scientz-100F) and then ground (30 Hz, 1.5 min) to powder form with a grinder (MM 400, Retsch). Then, 50 mg of each sample was added to 1,200 μL 70% (v/v) methanol pre-chilled to −20°C. Extracts were vortexed once for 30 s every 30 min, for a total of 6 times. After centrifugation at 10,000 g for 3 min (4°C), the supernatant was transferred to a new tube, filtered using a microporous membrane (0.22-μm pore size) and stored in an injection vial for UPLC-MS/MS analysis. The extracts were analyzed using an UPLC-ESI-MS/MS system (UPLC, ExionLC AD, https://sciex.com.cn/; MS, Applied Biosystems 4500 Q TRAP, https://sciex.com.cn/). The analytical conditions were as follows: UPLC column, Agilent SB-C18 (1.8 μm, 2.1 mm * 100 mm). L-2-chlorophenylalanine was used as the internal standard (Burlingway, Cas No: 103616-89-3, 1 ppm). The mobile phase consisted of solvent A, pure water with 0.1% (v/v) formic acid, and solvent B, acetonitrile with 0.1% (v/v) formic acid. Sample measurements were performed with a gradient program with starting conditions of 95% A, 5% B. Within 9 min, a linear gradient to 5% A, 95% B was programmed, and a composition of 5% A, 95% B was kept for 1 min. Subsequently, a composition of 95% A, 5.0% B was adjusted within 1.1 min and kept for 2.9 min. The flow velocity was set as 0.35 mL per min; The column oven was set to 40°C; The injection volume was 4 μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS. For ESI-Q TRAP-MS/MS analysis, the ESI source operation parameters were as follows: source temperature 550°C; ion spray voltage (IS) 5500 V (positive ion mode)/–4500 V (negative ion mode); ion source gas I (GSI) 50 psi; gas II (GSII) 60 psi; curtain gas (CUR) 25 psi; collision-activated dissociation (CAD) high. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to medium. DP (declustering potential) and CE (collision energy) for individual MRM transitions was done with further DP and CE optimization. A specific set of MRM transitions was monitored for each period according to the metabolites eluted within this period. Both hierarchical cluster analysis (HCA) and Pearson's correlation coefficient (PCC) analysis of the samples and metabolites were carried out using the ComplexHeatmap package in R. For HCA, a color spectrum was used for normalizing the signal intensities of the metabolites. Variable importance in projection (VIP) scores >1 and absolute Log_2FC ($|Log_2FC| \geq 1.0$ were used to determine differentially abundant metabolites. The VIP values were extracted from the result of Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA).

Genes with transcript levels or metabolites with contents that showed an absolute FC ≥ 2 with a false discovery rate (FDR) of 0.05 were regarded as differentially expressed genes (DEGs) or differentially abundant metabolites, respectively. KOBAS software[80] was used to determine the statistical enrichment of terms from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Significantly enriched KEGG pathways were defined with an FDR of 0.05 as threshold. The analysis of the rhizosphere microbiome was conducted using the Uparse algorithm (Uparse v7.0.1001, http://www.drive5.com/uparse/)[81] to cluster all effective tags across all samples. By default, sequences were clustered into operational taxonomic units (OTUs) with at least 97% identity. Representative OTU sequences were selected. The sequence with the highest frequency in OTUs was selected as the representative sequence of OTUs and then annotated with the species name by Mothur method and SILVA138 (http://www.arb-silva.de/) (with a threshold set to 0.8–1).[82,83] For determination of phytohormone contents, the samples were placed in a lyophilizer (Scientz-100F) and then ground (30 Hz, 1.5 min) to a powder with a grinder (MM 400, Retsch). Then, 50 mg (fresh weight) of each sample was extracted using methanol:water:formic acid (15:4:1, v/v/v). Under a nitrogen gas stream, the extracts were evaporated to dryness, followed by reconstitution in 80% (v/v) methanol and filtration with polytetrafluoroethylene with a pore size of 0.22 μm. The QTRAP 6500 LC-MS/MS platform (SCIEX, Macclesfield, UK) was used to analyze the extracts of each sample. The qualitative analysis of sample data was based on a standard sample database (Metware Biotechnology, MWDB). Quantification was performed through the multiple reaction monitoring mode (MRM) of triple quadrupole mass spectrometry.

Physiological and biochemical tests

The proportion of disease spots on peanut leaves was scored in ImageJ.[84] To score the refusal of *S. litura* to eat leaves,[79] the leaves of 4- to 5-week-old peanut plants (n = 18) were fully sprayed with a 1,000x diluted

GZM solution (corresponding to 1.3 mM lauryl alcohol, 123.7 μM 1-hexadecanol, and 23.3 μM Tween 80) by manual atomizing spray. Leaves sprayed with sterile water served as the control (CK) groups. Leaves were sprayed again 48 h later. After 72 h from the first spraying, six leaves with similar status per plant were collected. Five *S. litura* third-stage larvae were placed into a feeding box without food for 2 h to induce starvation. Six leaf blades for the treatment group and six blades for the CK (water) group were placed in far corners of the feeding box at the same time. After 12 h of feeding under dark conditions, chewing marks on the leaves were recorded and converted to a five-level scale (level 1, leaves were not eaten; level 2, slight feeding traces and 1–2 feeding plaques; level 3, slight feeding traces and 3–4 feeding plaques; level 4; 4–5 feeding traces, with obvious feeding traces; level 5, clear outline and large area of feeding plaques, and more than 5 feeding traces). Six biological replicates were conducted, and the experiments were carried out as three independent biological repeats. The contents for proline and malondialdehyde (MDA) in fresh leaves were determined with kits (proline, A107-1-1; MDA, A003-1; Nanjing Jiancheng Bioengineering Institute). For the determination of SOD and POD enzyme activities in fresh leaves, kits A001-3 (SOD) and A084-3 (POD) were used (Nanjing Jiancheng Bioengineering Institute). A catalase (CAT) activity assay kit (D799598, Sangon Biotech) was used.

Scanning electron microscopy

The leaves of 3-week-old peanut seedlings (n = 12) were fully sprayed with a 1,000x diluted GZM solution (corresponding to 1.3 mM lauryl alcohol, 123.7 μM 1-hexadecanol, and 23.3 μM Tween 80) by manual atomizing spray. Leaves sprayed with sterile water served as the control (CK) groups. Leaves were sprayed again at 5 and 7 weeks after seed germination. Then, leaves with similar status per plant were collected 1 week after the third spraying, immersed immediately in fixative solution (G1102-100 ML, Servicebio) for 2 h at room temperature, then transferred to 4°C for preservation. Tissues were washed with 0.1 M phosphate buffer (PB, pH 7.4) 3 times for 15 min each. Then tissues were transferred into 1% (w/v) OsO_4 in 0.1 MPB (pH 7.4) for 1–2 h at room temperature. Tissues were then washed in 0.1 MPB (pH 7.4) 3 times for 15 min each. The samples were gradually dehydrated through a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, 100%, 100%, all v/v) for 15 min each time and isoamyl acetate for 15 min. Samples were dried with a critical point dryer, followed by sputter-coating with gold for 30 s. Photographs were taken on an SEM Hitachi SU8100.

QUANTIFICATION AND STATISTICAL ANALYSIS

Unless stated otherwise, at least three replicates were conducted per experiment, and representative results are presented. Statistical analysis was performed in Microsoft Excel (version Office 365) and GraphPad Prism (version 8.02). Levene's and Shapiro-Wilk tests were used to assess group homoscedasticity and normality, respectively. Statistical significance was set to $p \leq 0.05$. Duncan's test were used to quantify statistically significant differences between datasets. All p-values are presented on the compared groups, with results considered statistically significant when $p < 0.05$.