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# Response of invasive weed *Mikania micrantha* to insect feeding

**Qilei Zhang[+], Guangxin Chen [+], Jundong Huang, Changlian Peng[*]**

School of Life Sciences, South China Normal University, Guangzhou 510631, China

[+] These authors contributed equally to this work.

[*]Corresponding author.

## Abstract

As an invasive plant, *Mikania micrantha* has lost the natural enemy of the country of origin in its invasion, but it will face the feeding of omnivorous insects. In order to explore the anti-insect-feeding defense strategy, *M. micrantha* with insect-feeding (IF) and non-insect-feeding (NIF) were selected as research materials. Results showed that contents of the defense substances were significantly higher in IF. However, net photosynthetic rate of IF leaves was significantly lower. As nutrient, the contents of sugar and protein were significantly lower in IF. Furthermore, the content of signal matter with indirect defense also increased significantly in IF. In addition, the expression of *FLOWERING LOCUS T* gene was up-regulated significantly in the IF *M. micrantha,* and it showed early flowering. Our results indicated that under insects feeding stress, *M. micrantha* can synthesize direct and indirect defense materials, and adopt the response strategy of early flowering.

**Keywords:** biological invasion; defensive capability; herbivorous insects; photosynthesis

# 1 Introduction

For the mechanism of the successful invasion of foreign plants, a number of studies have been carried out by national scientists, and many theories or hypotheses have been put forward [1], and enemy release hypothesis is one of them. The hypothesis is that some foreign plants can develop as invasive plants because of they get rid of the natural enemy that can be co-evolved with their origin [2]. Since invasive plants have lost their natural enemies of origin, resources can be re-allocated between defence and growth, and more resources are used to grow, resulting in invasive plants exhibiting excellent competitiveness in the genotype [3]. However, the invasive plants have lost their native-specific natural enemies in the invasion, but they will be threatened by the omnivorous natural enemy [4]. As a result, invasive plants also need some defensive strategies to deal with the damage of the omnivorous insects.

The insect resistance of plants can be divided into constituent type and induced type [5]. Constituent insect resistance defense response refers to plants blocking insect feeding or pathogen infection through their own inherent characteristics, such as plant thorns, epidermis fur and so on. Induced insect resistance defense response refers to the change of defense genes or characteristics after plants are damaged by insect, which makes it difficult for insects to eat further. Plant induced insect resistance defense response can also be divided into direct defense and indirect defense. Direct defense refers to the direct action of defense substances produced by physiological changes on phytophagous insects, resulting in their death or hindering their growth and development [6]. Toxic secondary chemicals mainly include alkaloids, flavonoids, lignin, tannins, non-protein amino acids and plant juvenile hormones, which can directly kill insects or affect the normal growth, development, feeding and reproduction of insects [7]. Plant induced insect resistance is an important aspect of plant resistance to phytophagous insects. The induced insecticidal response of plants begins with the recognition of phytophagous insects specific elicitors or other phytophagous insects related model molecules. After recognition, several signaling pathways, such as salicylic acid (SA), jasmonic acid (JA), hydrogen peroxide ($H_2O_2$) and ethylene (ETH), plant mitogen-activated protein kinase are activated. It leads to the increase of defense gene expression, the accumulation of defense compounds and the increase of volatile matter release, and finally makes plants show insect resistance [5, 8].

*Mikania micrantha* H. B. K., belonging to the *Asteraceae* family, is a fast-growing perennial creeping vine indigenous to Central and South America. At present, *M. micrantha* was listed as one of the 100 most harmful invasive plants [9]. The rapid spread of *M. micrantha* has

caused great damage to ecological balance, forestry and agriculture in invaded areas [10]. *M. micrantha* formed some strategies to adapt to the environment in its invasion [11]. However, in the field, many *M. micrantha* plants were eaten by insect. This study aimed to elucidate the defensive strategies of *M. micrantha* for insect feeding.

## 2 Materials and Methods

### *2.1 Plant materials*

In August, *M. micrantha* materials in this experiment were planted in the biological garden of South China Normal University, Guangzhou, China. In October, insect-feeding (IF) *M. micrantha* and non-insect-feeding (NIF) *M. micrantha* in the field were used as the research materials.

### *2.2 Total phenols determination*

Method described by Ainsworth et al. [12] was used to determine the total phenols. 0.05 g fresh sample of *M. micrantha* was weighed in 1 mL of 95% Methanol and extracted for 24 h at 4°C. The 0.5 mL sample was mixed with 1 mL of 10% Folin-Ciocalteu and 2 mL 0.7 M $Na_2CO_3$. The absorbance was measured at 765 nm by *UV-2450* spectrophotometer (*Shimadzu*, Tokyo, Japan). The standard curves were made with the different concentration of Gallic acid.

### *2.3 Flavonoids determination*

Method described by Zhang et al. [11] was used to determine the flavonoids. 0.05 g fresh sample of *M. micrantha* was weighed in 1 mL of 95% Methanol and extracted for 24 h at 4°C. The 0.15 mL sample was mixed with 1.85 mL purified water, 0.2 mL 5% $NaNO_2$, 0.3 mL 10% $AlCl_3$ and 1 mL 1 M NaOH. The absorbance was measured at 510 nm by *UV-2450* spectrophotometer (*Shimadzu*, Tokyo, Japan). The standard curves were made with the different concentration of Catechin.

### *2.4 Total antioxidant capacity determination*

The determination of the total antioxidant capacity (TAC) was according to the method described by Saha et al. [13]. 0.05 g fresh sample of *M. micrantha* was weighed in 1 mL of

95% Methanol and extracted for 24 h at 4°C. The 0.1 mL sample was mixed with 2.9 mL 120 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm by *UV-2450* spectrophotometer (*Shimadzu*, Tokyo, Japan). The standard curves were made with the different concentration of DPPH.

### *2.5 Tannin determination*

0.05 g fresh sample of *M. micrantha* was weighed in 5 mL of 70% Methanol and extracted for 24 h at 25°C. The 0.5 mL sample was mixed with 3 mL 4% Vanillin (4 g Vanillin dissolved in 100 mL Methanol), and 1.5 mL HCl and then bathed without light at 20°C for 20 min. The absorbance was measured at 510 nm by *UV-2450* spectrophotometer (*Shimadzu*, Tokyo, Japan). The standard curves were made with the different concentration of Catechin.

### *2.6 Salicylic acid, jasmonic acid, ethylene and $H_2O_2$ determination*

Fresh stems of *M. micrantha* (0.1 g) were weighed and ground with 0.5 mL PBS (50 mM pH = 7.3) on ice and placed at 4°C for 2 h. The grinding solution was centrifuged at 4°C for 20 min at 5,000 × *g*, and the supernatant was used to detect the contents of SA, JA, ETH and $H_2O_2$ using plant ELISA Kit (*Zike*, Shenzhen, China).

### *2.7 Soluble sugar determination*

The leaves and stems of *M. micrantha* were washed with purified water, dried at 70°C after 105°C for 20 min, sieved by 40 mesh sieve after grinded. The sample was extracted in a centrifugal tube containing 10 mL of distilled water, bathed in water at 80°C for 90 min, 0.04 g activated carbon was added, decolorized at 80°C for 30 min, and the filtered filtrate was used for the next experiment. The 1 mL sample was mixed with 10 mL 0.15% Anthrone-Sulfuric acid (0.15 g Anthrone dissolved in 100 mL 70% Sulfuric acid), and then bathed in water at 90°C for 15 min. The absorbance was measured at 620 nm by *UV-2450* spectrophotometer (*Shimadzu*, Tokyo, Japan). The standard curves were made with the different concentration of glucose.

### *2.8 Gene expression analysis*

TRIzol reagent (*Invitrogen*, California, MA, USA) was used to extract the total RNA by the steps on its manufacturer's instructions. Complementary DNA was synthesized using TopScript™ RT DryMIX (dT18) (*Enzynomic*, Daejeon, Korea) by the steps on the manufacturer's instructions. The relative expression of *FLOWERING LOCUS T* (*FT*) gene was detected by *Bio-Rad CFX96 Real-Time PCR System* (*CFX96*, *Bio-Rad*, California, USA) using a SYBR Premix Ex Taq™ II Kit (*Takara*, Tokyo, Japan). *Actin* was used as a reference gene; the primer pairs for which were 5′-TGAAATACCCCATTGAGCATGG-3′ (forward) and 5′-GAATCCAGTACAATACCTGTGGTAG-3′ (reverse). The primers for *FT* gene were as follows: 5′-TGTCGCATAGGGAGAGGGAT-3′ (forward) and 5′-TTAGCATGCATCCGTTGGCA-3′ (reverse). The relative gene expression was calculated by the $2^{-\Delta\Delta CT}$ method [14].

### *2.9 Microscopic observation of stem*

Stems of *M. micrantha* were fixed by the solution contained 2.5% glutaraldehyde and 2% polyformaldehyde) at 4°C for 12 h. Stems were stepwise dehydrated with different concentrations of alcohol (30–100%). Then, the critical-point-dried (using $CO_2$) stems were sprayed with 30 nm gold. The phenotypic characteristics of the stems were observed by scanning electron microscopy (SEM) (*Q25*, *FEI*, Oregon, USA).

### *2.10 Soluble protein and Rubisco determination*

Soluble protein and Rubisco was determined according to Zhang et al. [15]. 0.1 g Fresh leaves were homogenized in 2 mL of grinding medium composed of 50 mM Tris-HCl (pH 7.8) buffer, and the homogenate was centrifuged at 13,000 × *g* for 10 min. Supernatant (50 μL) was added to an equal volume of sample buffer sample buffer (pH 7.6), and then incubated in boiling water for 5 min. The samples (15 μL) were loaded onto gels containing 12.5% (w/v) resolving polyacrylamide gel and 4% (w/v) stacking polyacrylamide gel. Gel staining with *Coomassie Brilliant Blue R-250* (*Sigma*, USA) was followed by destaining overnight with gentle shaking to make sure that its background turned colorless. The molecular mass of the Rubisco large (RL) and small subunit (RS) is 55 and 15 kDa, respectively. The SDS-PAGE gel was scanned using the *GelDoc-It Imaging System* (*UVP*, Upland, CA, USA).

The supernatant was collected and diluted for 50 times. Then 250 μL of diluted supernatant was added into the same volume of Bradford solution. After 5 min, the absorbance was read at 595 nm by *UV-2450* spectrophotometer (*Shimadzu*, Tokyo, Japan). The standard curves were made with the different concentration of bovine serum albumin.

### *2.11 Measurement of Chl content*

0.1g of fresh material was extracted in 10 mL of 80% acetone at 4°C for 24 h. Determination of the absorbance value of the extract at 663 and 645nm by *UV-2450* spectrophotometer (*Shimadzu*, Tokyo, Japan). The Chl content was calculated according to the methods described by Wellburn [16].

### *2.12 Measurement of gas exchange parameters*

The portable measuring instrument *LI-6800* (*LI-COR*, *Inc*., USA) were used to measure the gas-exchange parameters of leaves in the morning (90:00–11:00) under synthetic PAR of 1,000 μmol(photon) $m^{-2}$ $s^{-1}$ with the ratio of red and blue light was set at 9:1. Parameters were recorded when the values were relatively stable.

### *2.13 Maximum photochemical efficiency determination*

The Chl fluorescence imaging system (*Technologica*, UK) was used to measure fluorescence. The minimum fluorescence ($F_0$) and the maximum fluorescence ($F_m$) of the leaves were measured using a 6,000 μmol(photon) $m^{-2}$ $s^{-1}$ saturating pulse after tue leaves were placed in the dark for 30 min. The maximum photochemical efficiency ($F_v/F_m$) of PSII was calculated as $F_v/F_m = (F_m - F_0)/F_m$ [17].

### *2.14 Data analysis*

The data were analysed using the software of *SPSS Statistics 19.0* (*IBM*, New York, NY, USA) by Student's *t*-test or Duncan's *post hoc* test at significance level $p < 0.05$. The data were plotted using the software of *SigmaPlot 12.5* (*Systat Software Inc*., Richmond, USA). The data were shown as the means ± standard errors (SE).

# 3 Results

## *3.1 Morphology characteristics of M. micrantha*

The phenotype of *M. micrantha* showed that under the pressure of insect feeding, the leaves of *M. micrantha* curl, the stem is thick and short, and flowered early (Fig. 1*A*). The transverse section of *M. micrantha* stem was observed by scanning electron microscope (Fig. 1*B*), the results were consistent with the statistical results. Insect feeding significantly increased the stem diameter of *M. micrantha* (Fig.1*C*).

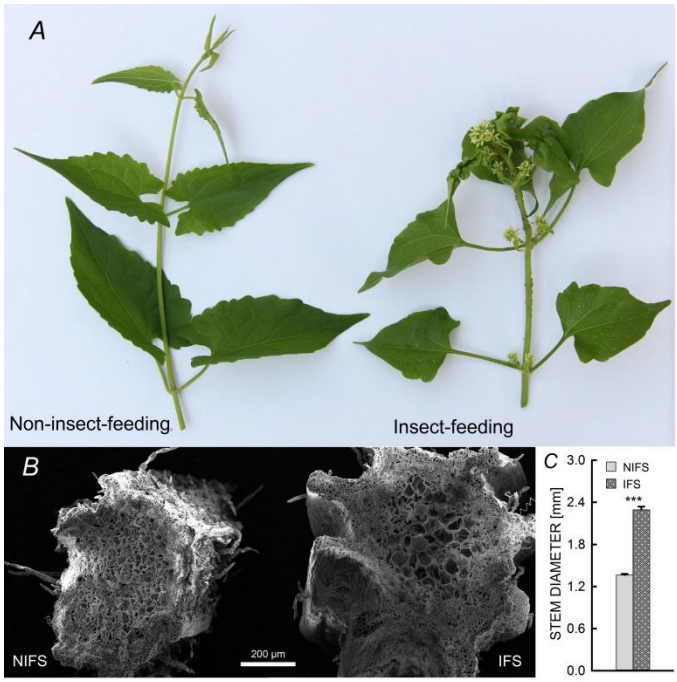


**Figure 1: The morphology characteristics of *Mikania micrantha*. Morphology characteristics of non-insect-feeding and insect-feeding *M. micrantha* (*A*). The picture of scanning electron microscope on stems (*B*) and stem diameter of *M. micrantha* (*C*) (*n* = 8). NIFS: non-insect-feeding stem. IFS: insect-feeding stem. Data are presented as the means ± SE (*n* = 8). Asterisks indicate different significant differences (*** *p*<0.001) according to two-sided Student's *t*-tests.**

## *3.2 Direct defense substance*

The content of total phenols, flavonoids and tannins in the *M. micrantha* increased significantly under insect feeding (Fig. 2*A*,*B*,*C*). The TAC of plants can be improved by antioxidants such as flavonoids and phenols. Results showed that TAC was significantly higher in the *M. micrantha* fed by insects (Fig. 2*D*). Sugar can be used as a source of nutrients and energy for insects, and the content of sugar decreased significantly when insects fed on it

(Fig. 2*E*).

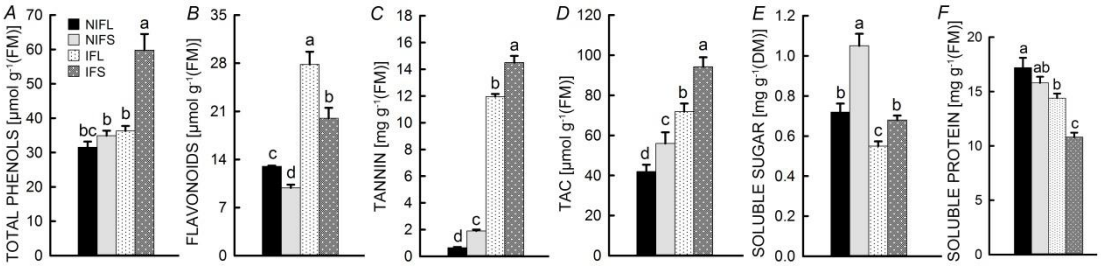


**Figure 2: The direct defense substance of *Mikania micrantha*. Contents of total phenols (*A*), flavonoids (*B*), tannins (*C*) and soluble sugar (*E*), soluble protein (*F*), and total antioxidant capacity (TAC, *D*) in the leaves and stems of insect-feeding and non-insect-feeding *M. micrantha* (*n* = 5). NIFL: non-insect-feeding leaf. NIFS: non-insect-feeding stem. IFL: insect-feeding leaf. IFS: insect-feeding stem. Data are presented as the means ± SE (*n* = 5). Different letters on the bar designate statistically reliable differences of the means at *p*<0.05.**

### *3.3 Signal defense substances*

In order to cause defense function in other parts, plants will produce signal materials to transmit information after being eaten. The results showed that the contents of SA, JA, ETH and $H_2O_2$ in the *M. micrantha* fed by insects increased significantly (Fig. 3*A*,*B*,*C*,*D*). SA, JA, ETH and $H_2O_2$ are involved in senescence and flowering regulation, which can promote the flowering of plants. The relative expression of *FT* gene in *M. micrantha* fed by insects is significantly higher (Fig. 3*E*), which is consistent with the flowering phenotype of *M. micrantha* in Fig. 1.

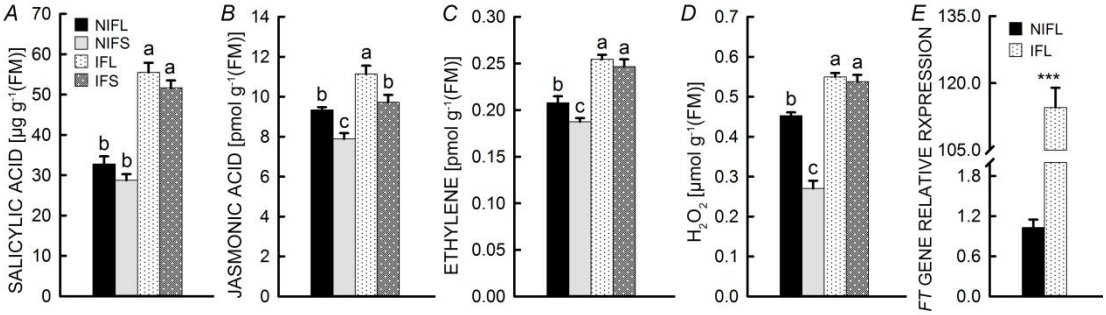


**Figure 3: The signal defense substance of *Mikania micrantha*. Contents of salicylic acid (*A*), jasmonate (*B*), ethylene (*C*) and $H_2O_2$ (*D*), and relative expression of *FLOWERING LOCUS T* (*FT*) gene (*E*) in the leaves and stems of insect-feeding and non-insect-feeding *M. micrantha* (*n* = 5). NIFL: non-insect-feeding leaf. NIFS: non-insect-feeding stem. IFL: insect-feeding leaf. IFS: insect-feeding stem. Data are presented as the means ± SE (*n* = 5). Different letters on the bar designate statistically reliable differences of the means at *p*<0.05. Asterisks indicate different significant differences (*****

**$p$<0.001) according to two-sided Student's $t$-tests.**

### *3.4 Photosynthetic capacity*

Photosynthesis is the basis of higher plant growth. Under insect feeding stress, the contents of Rubisco and Chl related to photosynthesis in the leaves of *M. micrantha* decreased (Fig. 4*A,B*), resulting in a significant decrease in the net photosynthetic rate ($P_N$) (Fig. 4*D*). The change trend of stomatal conductance ($g_s$) and transpiration rate ($E$) was the same as that of $P_N$, and it decreased significantly under insect feeding (Fig. 4*E,F*). However, the content of intercellular $CO_2$ concentration ($C_i$) in the leaves of *M. micrantha* increased significantly, which was fed by insects (Fig. 4*G*). The $F_v/F_m$ of the leaves also decreased significantly under insect feeding (Fig. 4*C*).

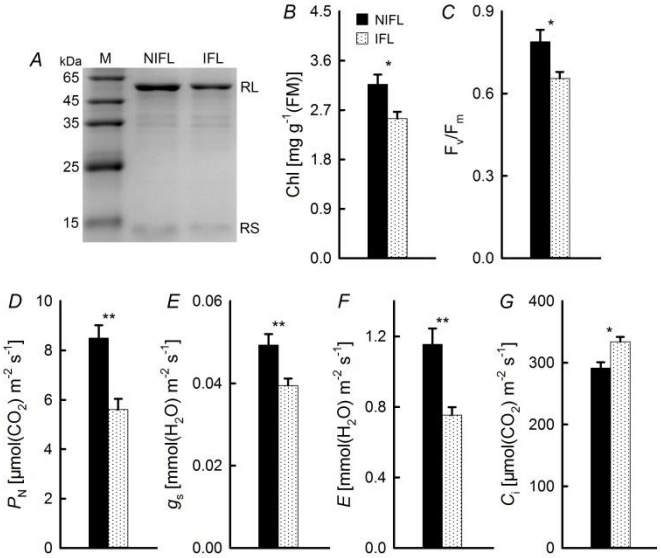


**Figure 4: The Photosynthetic capacity of *Mikania micrantha* leaves. Rubisco large (RL) and small subunit (RS) of *M. micrantha* leaves (*A*). The content of chlorophyll (Chl) in *Mikania micrantha* leaves (*B*). The maximum photochemical efficiency ($F_v/F_m$) of *M. micrantha* leaves (*C*). Gas exchange parameters of *M. micrantha* leaves, including the net photosynthetic rate ($P_N$, *D*), stomatal conductance ($g_s$, *E*), transpiration rate (*E*, *F*), and intercellular $CO_2$ concentration ($C_i$, *G*) ($n$ = 6). NIFL: non-insect-feeding leaf. NIFS: non-insect-feeding stem. IFL: insect-feeding leaf. IFS: insect-feeding stem. Data are presented as the means ± SE ($n$ = 6). Asterisks indicate different significant differences (* $p$<0.05, ** $p$<0.01) according to two-sided Student's $t$-tests.**

## 4 Discussion

Under insect feeding, the leaves of *M. micrantha* appear curly, and the stems become thicker and shorter. The content of toxic secondary chemicals in leaves and stems increased, while the content of soluble sugar decreased. *M. micrantha* is a native plant of Central and South America. Although getting rid of the natural enemies of the original place, it will face the feeding threat of the omnivorous insects in the invasion region. Under the pressure of insect feeding, the leaves of plants will appear deformity, which has been reported in the study of tea plants [18]. The results of this study show that the leaves of *M. micrantha* fed by insects also appear curly and the stems become thick (Fig. 1). In order to prevent insects from further feeding, the content of total phenols, flavonoids and tannins in leaves and stems increased significantly, and the contents of soluble sugar and protein, as the nutrition and direct energy source of insects, decreased significantly (Fig. 2). Phenolic compounds are important disease and insect resistant substances, mainly in the form of flavonoids and tannins. The content of phenolic compounds in plants can be used as indicators of chemical defense. After insect feeding, the total phenols content in plants will increase significantly, which will affect the development of insects [7]. Flavonoid can affect the growth and development of insect by inhibiting the beneficial bacteria in insect intestines. Tannins can lead to damage of midgut cells and loss of regeneration ability in insects, and have significant defensive effects on aphids, red spiders and whitefly. In the study on *Betula platyphylla* and *B. ermanii*, it was found that higher tannins and phenolic compounds were beneficial to avoid being eaten by insects [19].

Under the stimulation of insect feeding, the content of signal chemicals in leaves and stems increased. In the process of preventing insect feeding, in addition to the above toxic secondary metabolites will directly act on insects, SA, JA, $H_2O_2$ and ETH can indirectly play a defensive role [20]. They can be used as signaling substances to cause toxic secondary metabolites in other parts of the plant, forming systematic defense. It was discovered that herbivorous insects can activate the JA pathway [21]. In *Arabidopsis thaliana*, the expression of the genes related to JA was up-regulated and the content of JA increased significantly under the stimulation of insects. Our result showed that content of JA was significantly higher in the leaves and stems of insect-feeding *M. micrantha* (Fig. 3), which was consistent with the results in the research on tomato plants [22]. Previous study showed that insect sensitivity to ETH mediated defense in rice [23]. Resistance of SA synthetic gene deficient *sid2-1* mutants *Arabidopsis* to chewing herbivores weakened [24]. The contents of ETH and SA in the *M. micrantha* were

significantly increased under the feeding of insects (Fig. 3). $H_2O_2$ is an essential part of plant stress responses and it was implicated in herbivory-induced responses. Previous studies have shown that wounding alone is not caused by plants to produce a large number of $H_2O_2$, but insect does [25]. Our results were consistent with previous studies, and the content of $H_2O_2$ in the *M. micrantha* fed by insects is significantly higher (Fig. 3). Similar results have been reported in tomatoes [26].

Under insect feeding, *M. micrantha* showed early flowering. The results showed that the contents of ETH, $H_2O_2$ SA and JA were significantly higher in insect-feeding *M. micrantha*. ETH and $H_2O_2$ as signal molecule can stimulate expression of senescence genes and regulate plant senescence [27,28]. In the study on rice, it was found that the $H_2O_2$ content in the leaves of early flowering line was significantly higher than that of wild-type, and it showed the phenotype of early flowering and senescence [15,29]. Changing the expression of ETH receptor ETR2 can change the flowering time of rice [27]. Exogenous application of JA did not lead to early senescence of JA insensitive mutants, but accelerated the senescence of wild-type *Arabidopsis* [30]. In the process of plant leaves senescence, the contents of Rubisco and Chl, and the $P_N$ will gradually decrease [15]. Previous study have shown that overexpression of the *FT* gene can promote early flowering in plants [31]. In sunflower, SA can induce the expression of *FT* gene and promote its early flowering [32,33]. The results showed that *M. micrantha*, which was fed by insects, had early flowering, and the expression of *FT* gene was significantly higher than that of the non-insect-feeding *M. micrantha* (Fig. 3). Flowering is an important life history character of plant development and offspring reproduction, which is controlled by internal and external factors [34]. When plants encounter adverse environment, they will show the phenomenon of early flowering, which is considered as a kind of stress response, and they will flower and bear fruit as soon as possible [35,36]. The early flowering of *M. micrantha* in this study may be a response to the stress of insect feeding.

## 5 Conclusion

In its invasion regions, under the stress of insect feeding, *M. micrantha* increased the direct defense of the formation of toxic secondary metabolites, and also increased the content of indirect defense substances of signaling class to form a systematic defense. Furthermore, under the biological stress of insect feeding, *M. micrantha* adopted the strategy of early

flowering and fruiting to accelerate the completion of the reproduction process of offspring, so as to reduce the adverse effects of biological stress.

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**Conflicts of Interest:** The authors declare that they have no conflicts of interest to report regarding the present study.

## References

[1] Catford, J. A., Jansson, R., Nilsson, C. (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions*, 15, 22-40.

[2] Keane, R. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends Ecology & Evolution*, 17, 164-170.

[3] González-Teuber, M., Quiroz, C. L., Concha-Bloomfield, I., Cavieres, L. A. (2017). Enhanced fitness and greater herbivore resistance: implications for dandelion invasion in an alpine habitat. *Biological Invasions*, 19, 647-653.

[4] Doorduin, L. J., Vrieling, K. (2011). A review of the photochemical support for the shifting defence hypothesis. *Phytochemistry Reviews*, 10, 99-106.

[5] Howe, G. A., Jander, G. (2008). Plant immunity to insect herbivores. *Annual Review of Plant Biology*, 59, 41-66.

[6] Lou, Y. G., Baldwin, I. T. (2003). *Manduca sexta* recognition and resistance among allopolyploid *Nicotiana* host plants. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 14581-14586.

[7] Wright, D. M., Jordan, G. J., Lee, W. G., Duncan, R. P., Forsyth, D. M., Coomes, D. A. (2010). Do leaves of plants on phosphorus-impoverished soils contain high concentrations of phenolic defence compounds? *Functional Ecology*, 24, 52-61.

[8] Xu, H. X., Qian, L. X., Wang, X. W., Shao, R. X., Hong, Y., Liu, S. S., Wang, X. W. (2019). A salivary effector enables whitefly to feed on host plants by eliciting salicylic acid-signaling pathway. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 490-495.

[9] Yang, Q., Ye, W., Deng, X., Cao, H., Zhang, Y., Xu, K. (2005). Seed germination eco-physiology of *Mikania micrantha* H.B.K. *Botanical Bulletin of Academia Sinica*, 46, 293-299.

[10] Zhang, L.Y., Ye, W. H., Cao, H. L., Feng, H. L. (2004). *Mikania micrantha* H.B.K. in China - an overview. *Weed Research*, 44, 42-49.

[11] Zhang, Q. L., Zhai, J. J., Chen, G. X., Lin, W., Peng, C. (2019). The changing distribution of anthocyanin in *Mikania micrantha* leaves as an adaption to low-temperature environments. *Plants*, 8, 456.

[12] Ainsworth, E. A., Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protocols, 2, 875-877.

[13] Saha, M. R., Hasan, S. M. R., Akter, R., Hossain, M. M., Alam, M. S., Alam, M. A., Mazumder, M. E. H. (2008). *In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn. *Bangladesh Journal of Veterinary Medicine*, 6, 197-202.

[14] Livak, K. J., Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25, 402-408.

[15] Zhang, Q. L., Ding, W. Q., Wei, Y. X., Peng, C. (2019). Exogenous ascorbic acid delayed leaf senescence of early flowering rice mutant FTL10. *Photosynthetica*, 57, 960-966.

[16] Wellburn, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, 144, 307-313.

[17] Oxborough, K., Baker, N. R. (1997). Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components-calculation of qP and Fv'/Fm'; without measuring Fo'. *Photosynthesis Research*, 54, 135-142.

[18] Tian, Y., Wang, H., Hou, J., Zhang, L., Zhang, Z., Cai, X. (2019). Occurrence and

distribution of *Apolygus lucorum* on weed hosts and tea plants in tea plantation ecosystems. *Insects*, 10, 167, 2019.

[19] Matsuki, S., Sano, Y., Koike, T. (2004). Chemical and physical defence in early and late leaves in three heterophyllous birch species native to northern Japan. Annals of Botany, 93, 141-147.

[20] Pieterse, C. M. J., vander Does, D., Zamioudis, C., Leon-Reyes, A., van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, 28, 489521.

[21] Verhage, A., Vlaardingerbroek, I., Raai-jmakers, C., Van Dam, N., Dicke, M., Van Wees, S. C., Pieterse, C. M. (2011). Rewiring of the jasmonate signaling pathway in *Arabidopsis* during insect herbivory. *Frontiers in Plant Science*, 2, 47.

[22] Fujimoto, T., Tomitaka, Y., Abe, H., Tsuda, S., Futai, K., Mizukubo, T. (2011). Expression profile of jasmonic acid-induced genes and the induced resistance against the root-knot nematode (*Meloidogyneincognita*) in tomato plants (*Solanum lycopersicum*) after foliar treatment with methyl jasmonate. *Journal of Plant Physiology*, 168, 1084-1097.

[23] Nahar, K., Kyndt, T., Nzogela, Y. B., Gheysen, G. (2012). Abscisic acid interacts antagonistically with classical defense pathways in rice-migratory nematode interaction. *New Phytologist*, 196, 901-913.

[24] Bruessow, F., Gouhier-Darimont, C., Buchala, A., Metraux, J. P., Reymond, P. (2010) Insect eggs suppress plant defence against chewing herbivores. *Plant Journal*, 62, 876-885.

[25] Leitner, M., Boland, W., Mithofer, A. (2005). Direct and indirect defences induced by piercing-sucking and chewing herbivores in *Medicago truncatula*. New Phytologist, 167, 597-606.

[26] Sagi, M., Davydov, O., Orazova, S., Yesbergenova, Z., Ophir, R., Stratmann, J. W., Fluhr, R. (2004). Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum*. *Plant Cell*, 16, 616-28.

[27] Wuriyanghan, H., Zhang, B., Cao, W. H., Ma, B., Lei, G., Liu, Y. F., Wei, W., Wu, H. J., Chen, L. J., Chen, H. W., Cao, Y. R., He, S. J., Zhang, W. K., Wang, X. J., Chen, S. Y., Zhang, J. S. (2009). The ethylene receptor ETR2 delays floral transition and affects starch accumulation in rice. *Plant Cell*, 21, 1473-1494.

[28] Juvany, M., Müller, M., Munné-Bosch, S. (2013). Photo-oxidative stress in emerging and senescing leaves: a mirror image? *Journal of Experimental Botany*, 64, 3087-3098.

[29] Fang, M., Zhou, Z., Zhou, X.. Yang, H.; Li, M.;, Li, H. (2019). Overexpression of *OsFTL10* induces early flowering and improves drought tolerance in *Oryza sativa* L. *Peer J*, **7**, e6422.

[30] He, Y., Fukushige, H., Hildebrand, D. F., Gan, S. (2002). Evidence supporting a role of jasmonic acid in *Arabidopsis* leaf senescence. *Plant Physiology*, 128, 876-884.

[31] Hayama, R., Agashe, B., Luley, E., King, R., Coupland, G. (2007). A circadian rhythm set by dusk determines the expression of *FT* homologs and the short-day photoperiodic flowering response in pharbitis. *Plant Cell*, 19, 2988-3000.

[32] Dezar, C. A., Giacomelli, J. I., Manavella, P. A., Ré, D. A., Alves-Ferreira, M., Baldwin, I. T., Bonaventure, G., Chan, R. L. (2011). HAHB10, a sunflower HD-Zip II transcription factor, participates in the induction of flowering and in the control of phytohormone-mediated responses to biotic stress. *Journal of Experimental Botany*, 62, 1061-1076.

[33] Miki, S., Wada, K. C., Takeno, K. (2015). A possible role of an anthocyanin filter in low-intensity light stressinduced flowering in *Perilla frutescens* var. *crispa*. *Journal of Plant Physiology*, 175, 157-162.

[34] Fornara, F., de Montaigu, A., Coupland, G. (2010). SnapShot: control of flowering in *Arabidopsis*. *Cell*, 141, 550-550.

[35] Wada, K.C., Yamada, M., Shiraya, T., Takeno, K. (2010). Salicylic acid and the flowering gene *FLOWERING LOCUST* homolog are involved in poor-nutrition stress-induced flowering of *Pharbitis nil*. *Journal of Plant Physiology*, 167, 447-452.

[36] Koshio, A., Hasegawa, T., Okada, R., Takeno, K. (2015). Endogenous factors regulating poor-nutrition stress-induced flowering in pharbitis: the involvement of metabolic pathways regulated by aminooxyacetic acid. *Journal of Plant Physiology*, 173, 82-88.