

Article

# Inhibitory Effects of Plant Trypsin Inhibitors *Msti-94* and *Msti-16* on *Therioaphis trifolii* (Monell) (Homoptera: Aphididae) in Alfalfa

Hailong Zhao <sup>1,2,†</sup>, Hidayat Ullah <sup>1,3,†</sup>, Mark Richard McNeill <sup>4</sup>, Guilin Du <sup>5</sup>, Kun Hao <sup>1</sup>, Xiongbing Tu <sup>1,\*</sup> and Zehua Zhang <sup>1,\*</sup>

<sup>1</sup> State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; zhao.hailong@live.com (H.Z.); shabkadar@yahoo.com (H.U.); haokun8611@foxmail.com (K.H.)

<sup>2</sup> College of Plant Protection, Shenyang Agricultural University, Shenyang 110161, China

<sup>3</sup> Department of Agriculture, The University of Swabi, Anbar-23561, Swabi, Khyber Pakhtunkhwa, Pakistan

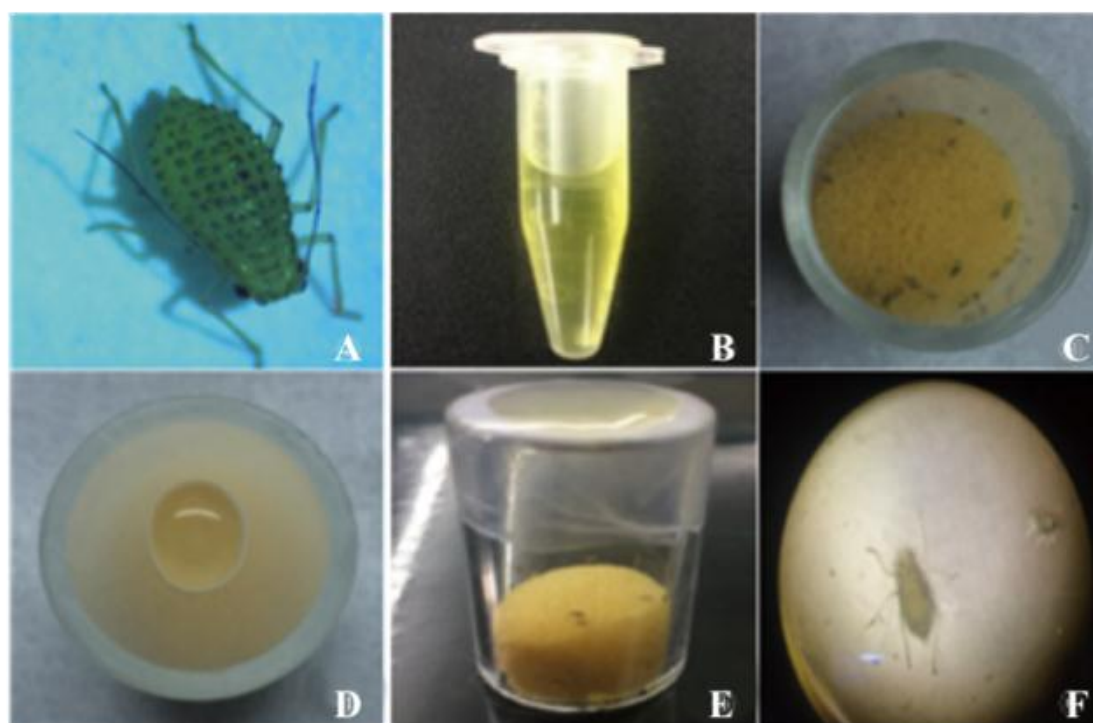
<sup>4</sup> AgResearch, Lincoln Research Centre, Christchurch 8140, New Zealand; mark.mcneill@agresearch.co.nz

<sup>5</sup> National Animal Husbandry Service, Beijing 100125, China; caasdgl@163.com

\* Correspondence: xbtu@ippcaas.cn (X.T.); zhangzehua@caas.cn (Z.Z.)

† These authors contributed equally to this study.

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**Figure S1.** Step-wise process of spotted alfalfa aphids reared with an artificial diet. (A) Healthy active spotted alfalfa aphid; (B) Aphid's artificial diet; (C) feeder with aphids sealed from the bottom with a sponge; (D) artificial diet poured on the first layer of Parafilm M; (E) sealed with another layer of Parafilm M from the top; (F) aerial view of aphids feeding.

**Table S1.** Equation of different enzyme curve along with regression value.

Enzyme	Equation	R <sup>2</sup>
Total proteinase	y=0.0025x+0.0822	0.9932
Trypsin	y=0.0002x+0.0642	0.9951
Chymotrypsin	y=0.0397x+0.0934	0.9910
Aminopeptidase	y=0.0239x+0.0964	0.9914

**Table S2.** Enzyme activities assays of aphids on different concentration of feed with *Msti-94* recombinant protein.

Concentration (µg/mL)		Enzyme (U/mg)			
		Total Proteinase	Trypsin	Chymotrypsin	Aminopeptidase
2000	Control	164.56±8.80a	2265.33±40.21a	11.58±0.39a	19.23±0.93a
	<i>Msti-94</i>	159.81±22.84a	2136.01±141.26a	9.78±0.77b	17.77±1.26a
3000	Control	169.59±10.90a	2252.93±62.07a	11.55±0.69a	19.33±0.98a
	<i>Msti-94</i>	156.52±14.45a	2096.15±177.30b	8.30±0.55c	17.87±0.50a
4000	Control	170.84±14.57a	2376.21±306.04a	11.07±0.54a	18.03±1.16a
	<i>Msti-94</i>	148.53±24.97b	1775.56±58.95c	8.37±0.59c	16.04±0.97b
5000	Control	173.19±7.49a	2247.07±148.25a	10.57±0.76a	18.32±0.20a
	<i>Msti-94</i>	114.00±5.71c	1561.47±109.28d	6.29±0.32d	11.74±1.19c
6000	Control	172.84±2.21a	2229.82±163.16a	10.81±0.75a	18.30±1.72a
	<i>Msti-94</i>	67.42±13.96d	981.57±99.38e	4.14±0.74e	8.67±1.22d

Data described in terms of "mean value ± standard error" at 0.05 level of significant ranged by Duncan analysis with SPSS 20.0.

**Table S3.** Enzyme activities assays of aphids on different concentration of feed with *Msti-16* recombinant protein.

Concentration (µg/mL)		Enzyme (U/mg)			
		Total Proteinase	Trypsin	Chymotrypsin	Aminopeptidase
2000	Control	174.66±14.33a	2314.24±107.64a	11.28±0.71a	7.39±1.15e
	<i>Msti-16</i>	151.58±17.38b	2169.88±302.27b	10.85±0.85a	17.58±0.90b
3000	Control	184.12±6.61a	2197.65±73.79b	10.88±0.50a	22.04±0.40a
	<i>Msti-16</i>	145.72±14.54b	2181.57±214.15b	9.63±0.42b	16.18±0.53bc
4000	Control	181.33±5.05a	2312.06±95.73a	10.77±0.75a	17.83±0.18b
	<i>Msti-16</i>	137.38±5.70c	1614.91±932.63c	8.37±0.59c	15.18±0.78c
5000	Control	171.99±22.47a	2251.09±291.00a	10.49±0.84a	18.26±1.60b
	<i>Msti-16</i>	115.00±7.77d	1260.64±304.80d	6.41±0.76d	11.91±1.01d
6000	Control	170.10±6.90a	2187.60±95.50a	10.90±1.04a	17.46±1.80b
	<i>Msti-16</i>	72.63±20.83e	913.07±176.25e	5.04±0.51e	11.26±1.15d

Data described in terms of "mean value ± standard error" at 0.05 level of significant ranged by Duncan analysis with SPSS 20.0.

**Table S4.** Enzyme activities assays of aphids on different feed with recombinant purified protein.

Treatments (800 µg/mL)	Enzyme			
	Total Proteinase (U/mg)	Trypsin (U/mg)	Chymotrypsin (U/mg)	Aminopeptidase (U/mg)
Control	235.64±9.04a	2903.18±168.20a	13.42±0.67a	22.06±0.51a
<i>Msti-94</i>	139.06±19.25b	1761.64±150.45b	9.13±1.14b	15.86±0.84b
<i>Msti-16</i>	137.18±16.80b	1687.94±104.17b	8.81±0.94b	15.11±0.83b