0112

Increased Fecundity in Strains of *Tribolium castaneum* (Herbst.) and *Sitophilus zeamais* (Motsch.) after Ten Generations of Selection with Methyl Bromide

C. J. Waterford ¹ and R. G. Winks²

Abstract: Five strains of *Tribolium castaneum* (Herbst.) and two strains of *Sitophilus zeamais* (Motsch.) were selected with methyl bromide for ten generations. Investigation into the reproductive capacity of the progeny compared with that of the parent strains showed increased fecundity in all strains of *T. castaneum* and in one of two strains of *S. zeamais*.

Changes in tolerance of the selected strains were only of the order of x1.3 at the LD_{50} . However, in quarantine and pre-shipment treatments with methyl bromide this increased fecundity has implications for the re-establishment of infestations in marginal' treatments thereby increasing the risk of fumigation failure. This could become an important consideration, for example, in fumigation of poorly sealed shipping containers.

Key words: fecundity, resistance, fitness, methyl bromide

Introduction

Methyl bromide remains the fumigant of choice for quarantine fumigation against insect infestations in international cargo [1]. Requirements to fumigate shipments with methyl bromide have taken on an added level of complexity with an estimated 400 million container movements occurring every year worldwide. This increased shipment of cargo in containers and the commercial belief that containers are gastight has created a weakness in the quarantine treatments that relies on fumigation with methyl bromide.

Ball and van S. Graver^[2] showed that reaching the required dose in unsheeted containers filled with hay for export is correlated with half life pressure decay time. When control of infestations to quarantine standards is the goal, data presented here indicate that the need for gastight enclosures with methyl bromide now becomes more important. This is emphasised by the recent suspension of fumigation of "tarpless containers" by USDA APHIS ^[3].

Materials and Methods

Test insects used were strains of *T. castaneum* and *S. zeamais* collected during the FAO survey of pesticide susceptibility during 1972 – 1973 by Champ and Dyte [4] and held in laboratory culture. Culturing and general handling techniques follow those described in Winks [5].

Selection followed the exposure protocols of the FAO resistance method for methyl bromide [6] with selection doses targeted approximately at 70% mortality. Survivors were cultured as parents of the next generation.

The concentration of methyl bromide was confirmed at 99.9% purity by gas chromatography using the response of a Gowmac (gas density detector. All dosing and handling was carried out at $25\,^{\circ}\mathrm{C}$. The insects were starved and conditioned in an incubator at $25\,^{\circ}\mathrm{C}$, $57\,^{\circ}\mathrm{M}$ r. h. overnight prior to exposure.

The parent and final selection for each strain was assessed for mortality response when exposed to a range of concentrations for a five hour exposure period in 2. 5 L desiccators. Groups of 200 adults were used at each exposure time. End-point mortality response was determined from successive observations using the method recommended by Winks [7] and results were analysed using the method of Finney [8]. Parameters of the response lines for strains tested are shown in Tables 1 and 2.

Fecundity of strains was assessed by culturing 200 parents in 150 g of wholemeal flour supplemented with a small amount of brewers yeast. Parents were removed after seven days oviposition and sexed. After six weeks progeny were removed and the total weight estimated from the weight of 200 parents. The number of progeny produced per female was calculated.

^{1.} CSIRO Entomology, GPO Box 1700, Canberra, ACT 2615, Australia

^{* [} colin. waterford@ csiro. au]

^{2.} PO Box 294, Calwell, ACT, 2905, Australia

Results and Discussion

Parameters of the response lines for strains tested are shown in Table 1 and changes in fecundity shown in table 2.

Though the levels of resistance developed over ten generations of selection are not large, up x1.6 for *T. castaneum*, this could impact on marginal fumigations in fumigation enclosures where leaks, combined with the prevailing ambient conditions, dilute the applied fumigant in critical areas such as near the doors of containers. This is highlighted by Ball and van S. Graver [2] who reported several containers between 10 and 20 seconds pressure half life pressure decay times that did not achieve the target Ct

product. Given that higher levels of resistance would lead to higher survival in these under dosed areas, and combined with the increased fecundity reported here (up to 193% for one strain of *S. zeamais* and 122% for *T. castane-um*) an unpleasant surprise may await when the doors of such a container are opened. Clearly this poses significant implications in quarantine treatments.

Acknowledgements

We thank Ms Avis Walton who provided technical assistance in culturing and mortality assessment and Jan van S. Graver for comments on this paper.

Table 1. Dosage estimates and parameters of regression of probit mortality on log dosage for methyl bromide selections of adults of *Tribolium castaneum* and *Sitophilus zeamais* at 25 $^{\circ}\text{C}$, 57 % r. h. with resistance changes at the LD₅₀ and LD₉₉ compared to the parent strain.

57 % 1. In with resistance changes at the DD 50 and DD 99 compared to the parent strain.								
Strain	LD ₅₀ mg. hr L ⁻¹	LD ₉₉ mg. hr L ⁻¹	Resistance compared to parent strain at		Slope	Mean probit	Heterogeneity	
			LD_{50}	LD_{99}		response(Y)	\mathbf{X}^2	d. f.
TC12	32.73	46.66			19.9	7.05	5.03	5
TC12m10	45.5	65.7	x 1.4	x 1.41	13.0	4.9	17.3	12
TC367m4	50.21	65.05			20.7	4.9	12.7	5
TC367m11	59.56	82.15	x 1.19	x 1.26	16.6	4.8	8.76	5
TC369	42.2	51.5			43.3	5.3	. 69	3
TC369m10	59.6	71.4	x 1.4	x 1.37	60.2	5.1	18.9	5
TC408	36.7	52.5			18.5	4.6	57	2
Tc408m14	63.25	84.5	x 1.7	x 1.6	15	5.6	34	2
TC411	41.6	56.4			17.6	5.2	5.8	5
TC411m10	51.6	70.0	x 1.24	x 1.24	17.6	4.9	20.6	5
SZM9	20.3	25.3			19.2	5.2	4.1	5
SZM9m10	26.8	32.6	x 1.25	x 1.22	21.3	5.1	36	5
SZM23	19.9	25.2			22.9	5.2	8.54	5
SZM23m10	26.6	32.1	x 1.27	x 1.21	28.7	5.3	16.6	5

Table 2. Comparison of fecundity of parent and methyl bromide selected strains of *Tribolium castaneum* and *Sitophilus zeamais* at 25 °C ,57 % r. h.

or recommendate suspensions were expensions at the expension of the second seco							
Strain	Sex ratio	Number o	% increase				
	₽/₺	Total	Per ♀	% increase			
TC12	119/81	2540	20.5				
TC12m10	103/97	2806	27.2	33			
TC367m4	111/88	1286	11.6				
TC367m11	97/102	1551	16	38			
TC369	107/93	2004	18.7				
TC369m10	97/100	1937	20	7			
TC408	96/104	2799	29.2				

Strain	Sex ratio	Number o	0/. :		
Strain	₽/\$	Total	Per ♀	% increase	
TC408m14	81/118	3047	37.6	29	
TC411	55/44	708	12.9		
TC411m10	48/53	1379	28.7	122	
SZM9	108/92	2799	25.9		
SZM9m10	106/94	1875	17.7	-33	
SZM23	107/93	1447	13.5		
SZM23m10	119/81	4703	39.5	193	

References

- [1] AQIS Part B Treatments and Fumigants AQIS Methyl Bromide Fumigation Standard Version 1.3 January 2008.4 +61 pp. Accessed on 5 July 2008 at: http://www.daff.gov.au/__data/assets/pdf file/0009/113499/atf part b.pdf
- [2] Ball S., van S. Graver J. E., 1997. Pressure tests to determine need for sheeting loaded freight containers before fumigation. In: *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products*, (Edited by Donahaye, E. J., Navarro, S. and Varnava, A.), 21 26 April 1996, Printco Ltd., Nicosia, Cyprus, 353 358.
- [3] USDA APHIS. Notice of Suspension of Tarpless Container Fumigations. Accessed on 5 July 2008 at: http://www.aphis.usda.gov/plant_health/spotlights/downloads/TarplessFumiga-

- tions. pdf
- [4] Champ B. R., Dyte C. E. 1976. Report of the FAO global survey of pesticides susceptibility of stored grain pests. In: FAO Plant Production and Protection Series No. 5. p. 297. Food and Agriculture Organization of the United Nations.
- [5] Winks, R. G., 1982. The toxicity of phosphine to adults of Tribolium castaneum (Herbst):
 Time as a response factor. Journal of stored products Research. 18, 159 169
- [6] Anon., 1975. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. 16. Tentative method for adults of some major species of stored cereals, with methyl bromide and phosphine. FAO Plant Protection Bulletin 23, 12 25
- [7] Finney, D. J., 1971. Probit Analysis, 3rd edn. Cambridge University Press