Resistance of psocid, *Liposcelis bostrychophila* Badonnel, (Psocoptera: Liposcelididae) and the stability to controlled atmosphere

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Abstract

The theme of the present paper is that adult populations of the psocid, *Liposcelis bostrychophila*, were reared on artificial diet of wholemeal wheat flour, skimmed milk powder and yeast (10 1 1) at 28℃, 80% rh, and exposed for 30 generations to an atmosphere containing 35% CO_2, 21% O_2, and 44% N_2, in order to select a strain resistant to controlled atmosphere (CA) Selection pressure was maintained at around 70% mortality At the 30th generation, comparison of sensitivity between the selected strain and the original susceptible strain (CA-S) indicated a resistance factor (RF) at the 50% mortality level (LT_{50}) of 5.6-fold, and this strain still has the genetic potential to develop resistance continuously Removal of selection pressure for 5 generations from 1 sub-populations of the selected strain to the 25th generation revealed that the resistance was unstable and the resistance partially retained. After 5 generations, the RF decreased by 63.2%

Introduction

Controlled atmosphere (CA) with elevated carbon dioxide or nitrogen and depleted oxygen can be effective in controlling insects and mites in stored grain (Jay, 1984; Annis, 1987; Jayas et al , 1991) Under airtight conditions, controlled atmosphere fumigation can be economical (White et al., 1993), leaves no chemical residues in the grain and preserves grain quality (Banks, 1981) In some applications, carbon dioxide can replace methyl bromide as a fumigant (Newton, 1993) to be used to control stored product insects in food commodities, particularly grain (Jay, 1984) and insects infesting museum artifacts (Burke, 1993). Recent developments in its use with the sheeted stack method indicate its values as an alternative 'non-toxic' control method of fumigants in long-term storage of bagged grain (Annis, 1990)

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In field populations of stored-product insects, resistance to the most commonly used fumigants such as phosphine, methyl bromide (Champ and Dyte, 1976; Ho and Winks, 1995; Leong and Ho, 1994) and ethylene dibromide (Bond, 1973) has been demonstrated It has resulted in serious limitations in their use for stored-product protection (Donahaye, 1990a) In a similar way, extensive use of CA in insect control programs might be expected to favor selection of strains resistant to hypercarbia and hypoxia (Donahaye, 1990a,b,c)

Banks and Annis (1977) made the first suggestion of the possibility that stored-product insects would develop resistance to CA Navarro and Calderon (1973) warned of the situations that might arise where insects survived around leaks in incompletely sealed structures. Bond and Buckland (1979) were the first to show that stored-product insects had the genetic potential to develop resistance to CA, when they obtained a 3-fold increase in tolerance to CO_2 by *Sitophilus granarius* (L) after selection for seven generations Navarro et al. (1985) examined the resistance of *Sitophilus oryzae* selected to both 40% and 75% CO_2 in air over seven and ten generations, respectively. The resistance factors they recorded were 2.2 and 3.3-fold. Donahaye (1990a, b) investigated the resistance of *Tribolium castaneum* selected to both 65% CO_2 and 0.5% O_2 over forty generations, the resistance factor of 9 2 and 5.2-fold were developed to resistant to hypercarbia and hypoxia, respectively He also revealed that the resistance obtained in the adult stage was conferred to other stages (Donahaye et al , 1992). More recently, Zhao and Zhang (1994) monitored the combined effects of carbon dioxide and oxygen deficiency on *Tyrophagus putrescantiae* over twenty-five generations under five CA environments and 3 or 5-fold resistance was obtained. All of these studies also revealed that the significant reduction would occurr in resistance after selection pressure was relaxed for five or four generations (Navarro et al., 1985; Donahaye, 1992a, b; Zhao and Zhang, 1994)

Psocid, *Liposcelis bostrychophila* Badonnel, is of worldwide distribution and frequently found in the stored-product (Turner, 1994). It is often very abundant in cereal stores in hot, humid regions (Lee et al , 1992). However,

L. bostrychophila is usually considered to be of minor significance as stored-product insect pests and generally of little economic importance (Mockford, 1993). From time to time, Psocids, *Liposcelis species*, have been found infesting grain in very large numbers (Rees, 1994). In a socio-economic survey of psocid infestation in Indonesia and India, it was concluded that psocid posed a small but real problem in the storage system (Kleih and Pike, 1995). After pest control treatments stores often become recolonized with psocids very rapidly (Santoso et al, 1996) The development of separate pest control measures against these pests would probably not be effective.

Based on the survey, *L bostrychophila* has developed to be one of the dominant pest species in CA treatment grain storage environment, which is mainly adopted at present in China. The effect of CA to *L. bostrychophila* has been studied by Leong and Ho (1994, 1995). They found that the adults of *L bostrychophila* rank among the more susceptible in comparison with other insects (Annis, 1987); However, the eggs of *L. bostrychophila* are among the more tolerant With increasing interest and infesting situation in *L bostrychophila*, the present study was undertaken not only to investigate whether the psocid is capable of developing induced tolerance to CA under laboratory conditions, but also to study the stability of resistance.

Materials and Methods

Insect

Liposcelis bostrychophila, a colony which has been established in 1990 since the nymphs were collected from a wheat store in Chongqing, China, was reared on artificial diet of whole wheat flour, skimmed milk and yeast powder (10 1 1) in laboratory at constant temperature of 28 ± 0.5℃, and relative humidity 80%, in dark conditions

Apparatus and the chosen atmosphere

A controlled atmosphere unit capable of generating gaseous compositions from three gas (CO_2, O_2 and N_2) in the range of 0 – 100% was used in this study. The unit consisted of gas cylinders and regulators, three flowmeters, a gas blender, an insect chamber with 24 exposure units constructed from Erlenmeyer flasks, and all gas concentration measurements were carried out with the same gas chromatography apparatus The whole apparatus, as described by Wu et al (1990), was kept in a constant temperature room at 28 ± 1℃

To assess the combined effect of carbon dioxide-enriched and oxygen deficiency on *L Bostrychophila*, a gas mixture used for selection consisted of 35% CO_2, 1% O_2 and 44% N_2. The high CO_2 and low O_2 concentration are similar to those obtained in commercial practice

Bioassay

The base-line sensitivity of the non-selected strain was determined at the 2nd generation by probit analysis; then sensitivity of both the CA-selected and the non-selected strains was determined at 5 generation steps The experimental procedure was as follows: six group of 100 adults 2 – 3 days old were placed in six flasks; five flasks were exposed to CA (35% CO_2, 1% O_2 and 44% N_2) over a range of five exposure times and the sixth flask served as control. Due to heterogeneity of response, each experiment was repeated 5 times, giving a total of 30 results At the end of each exposure time the flasks were removed from the apparatus, and were held at 28℃ and 80% rh for 48 hours, at which time mortality counts were made

According to the results of bioassay, the log-time against probit-mortality lines was developed, and the comparison of mortalities between the strains was made by comparing the times required for the CA mixture to produce 50% kill (LT_{50})

Exposure techniques

An adult population of psocid were exposed for 30 generations to CA (35% CO_2, 1% O_2 and 44% N_2). Selection pressure was maintained around 70% mortality after each exposure, dead and surviving insects were counted samplely to determine the level of selection pressure and survivors were used for breeding the following generations.

Stability of increased resistance to CA

To determine the stability of the induced tolerance to CA, one sub-population of CA-selected strain was separated at the 26th generation. Exposure to CA was discontinued for 5 generations, and then response to CA was re-evaluated at each generation and compared with the continuously selected CA strains and the non-selected strain.

Results

Resistance development of *L. bostrychophila* to CA over 30 generations

Non-selected generations showed stability in their response as expressed in the time required to produce 50% mortality at 35% CO_2 and 1% O_2, so the base-line sensitivity was obtained by probit analysis at the 2nd generation The exposure time required to obtain LT_{50} values for successive selected generations of *L. bostrychophila* adults exposed to CA is shown in table 1.

According to Navarro et al. (1985), 'low levels of resistance recorded in stored products insects exposed to modified atmospheres has been referred to as tolerance' Since the resistance levels obtained in this study with *L*

bostrychophila exposed to CA were also low, the term tolerance was used.

The calculated tolerance factors (based on LT_{50}) for *L. bostrychophila* exposed to 35% CO_2 and 1% O_2 were increased to 5.6-fold at the 30th generation Up to the 30th generation there was a general trend of increased tolerance to the CA. At the 30th generation there was still no indications of CA-selected strain that the induced tolerance had peaked.

Stability of increased resistance to CA

The stability of resistance of CA-selected strain when selection pressure was relaxed is given in Table 2. The results showed that the resistance was unstable and the resistance partially retained. After 5 generations, the TF values decreased 63.2%. It declined rapidly from the 1st generation to the 4th generation, while from the 4th to the 5th generation it stabilized gradually when selection was suspended.

Table 1. Response of selected generations of *L. bostrychophila* adults exposed to CA.

F_x	Regression lines[a] ($Y = a + bx$)	LT_{50} (h.)	Tolerance factor[b] (F_x/F_0)
F_0	$-2.1628+8.8104\,x$	6.5012	1.0
F_5	$-0.9598+5.0539\,x$	15.1094	2.3
F_{10}	$-1.5333+4.9205\,x$	21.2702	3.3
F_{15}	$-2.0298+4.9874\,x$	25.6751	3.9
F_{20}	$-3.0241+5.3542\,x$	31.5250	4.8
F_{25}	$-3.6073+5.6931\,x$	32.5000	5.0
F_{30}	$-3.0997+5.1782\,x$	36.6600	5.6

[a] Regression line represents the relationship between the probit-mortality(Y) and the log-times (x).

[b] Based on LT_{50} values Same as follows

Table 2. Effect of suspending selection pressure on the tolerance of *L. bostrychophila* to CA

F_x	Regression lines ($Y = a + bx$)	LT_{50} (h)	Tolerance factor (F_x/F_0)	Loss in TF(%)
F_{25}	$-3.6073+5.6931\,x$	32.5000	5.0	–
F_{26}	$-2.5227+5.5201\,x$	23.0560	3.5	29.0
F_{27}	$-1.7017+5.2715\,x$	18.7211	2 9	42.4
F_{28}	$-0.9028+4.9712\,x$	15.3957	2.4	52.6
F_{29}	$-0.4851+5.0749\,x$	12.0456	1.9	62.9
F_{30}	$-0.5004+5.1015\,x$	11.9727	1.8	63.2

Discussion

The consistently low slope of the log-probit regression lines of time against mortality showed that the high heterogeneity of response continued throughout the CA-selected strains. The expected progressive steepening of the probit line as CA-selected populations became more homogeneous, did not occur. At the 30th generation the slope for the CA-selected strain was low, indicating that the slope had remained essentially unchanged throughout the series of selection. These findings are similar to those of Donahaye (1990), in his work on selected resistance to CA of *Tribolium castaneum*. He concluded that the resistance to CA was influenced by the multiple genetic factors, each factor had a relatively small effect but the general results was to produce a population with improved ability to withstand the effects of the controlled atmosphere.

The resistance of the CA-selected strain was unstable when selection pressure was relaxed This result is fully consistent with Donahaye (1990), Navarro et al. (1985), Zhao and Zhang (1994) Since resistance appears to be polyfactorial and their potential haven't exhausted at the 30th generation, it is suggested that stability of resistance may become even greater as the population become more homogeneous

In conclusion, the above results indicated that *L. bostrychophila* has genetic potential to develop resistance to CA. The suspension of selection caused a considerable reduction in the tolerance factors In view of the low rate of development of resistance exhibited by *L. bostrychophila* and the fact that under field conditions CA-treated insect strains are likely to mix with non-treated populations, the risks of endangering the practical application of CA are greatly reduced. Therefore, it would be advisable to integrate the CA technique with other control methods, to minimize the risk of selection of resistance to CA over successive generations Furthermore, care should be taken that CA is not used in situations where the treatment is likely to be incompletely treatment, so as to minimize selection of CA-resistance strains. Since this is a non-toxic residue treatment, an increase in CO_2 concentration and decrease in O_2 concentration, and extension of exposure time, would not have a harmful effect on the treated commodities.

It should also be noted that the selections were not carried out by using gas concentration or a relative humidity occurring in real-life situations. But they were chosen to reduce the influence of the additional factors of desiccation, in order to obtain a strain resistant to CA upon which the mechanisms of resistance could be examined in subsequent studies Clearly, these findings cannot be used as guidelines for predicting the development of resistance in the field.

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