Adler C, Murray A (2012) Taking the tropics home: Controlled atmospheres for the control of tobacco beetles in large-scale testing. In: Navarro S, Banks HJ, Jayas DS, Bell CH, Noyes RT, Ferizli AG, Emekci M, Isikber AA, Alagusundaram K, [Eds.] Proc 9th. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products, Antalya, Turkey. 15 – 19 October 2012, ARBER Professional Congress Services, Turkey pp: 488-494

TAKING THE TROPICS HOME: CONTROLLED ATMOSPHERES FOR THE CONTROL OF TOBACCO BEETLES IN LARGE-SCALE TESTING

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ABSTRACT

For years phosphine fumigation has been used for pest control in tobacco. Problems arose with the development of resistant insect strains. Moreover, toxic fumigants are potentially hazardous to man. This is why a consortium of interested tobacco companies, the CORESTA subgroup Pest and Sanitation Management in Stored Tobacco, decided for a large-scale testing of controlled atmospheres (CAs) at practical conditions. In most tobacco producing countries warm product temperatures favour the efficacy of hypoxic atmospheres and allow for sufficiently short treatment times. To obtain similar treatment times in Central Europe it was decided to heat the incoming tobacco prior to CA application. Samples of all stages of tobacco beetles were added to tobacco bales in order to monitor the efficacy of the treatment. Data loggers for temperature and r.h. were added to the samples to record physical conditions during transportation and treatment. Untreated control samples accompanied the experimental samples. One of the treatment chambers was a gastight cube measuring 10m in each direction. Practical treatments proved difficulties to reach uniform high temperatures in such a chamber. In some cases cold winter temperatures on the outside of the chamber led to massive condensation of water from tobacco leaves. Eggs, various larval stages, pupae, and adult beetles could be controlled with a hypoxic CA containing some 0.5% oxygen at temperatures of 28°C and within 9 days of exposure time. Few survivors were recorded, when a similar CA was tested at 38°C and 2.5 days exposure time.

Key words: tobacco, storage, disinfestation, *Lasioderma serricorne*, controlled atmospheres.

INTRODUCTION

Fumigants have been used for many years as the primary measure for insect pest control in large and commercial storages of dry stored products. In many regions of the world phosphine has been one of the fumigants heavily relied upon. This is still true in most cases even though some difficulties have arisen over the last decades: Continuous fumigation with just phosphine at low dosages (e.g. siro flow, J-system), competition for low costs and fast

treatment times, and low-quality sealing gave rise to the development of phosphine-resistant insect strains (Collins et al. 2003, Nayak et al. 2010). Moreover, phosphine was also used in-transit by untrained seamen or in rural areas by not sufficiently educated laymen and has caused a number of casualties by faulty use or abuse. Metal phosphides that develop phosphine in the presence of humidity sometimes gave surprising results that caused hazards when too high moisture contents caused too high PH_3-doses and ignition or when too low moisture contents let to insufficiently low dosages for pest control and caused problems when not completely degassed product had to be discarded. Cases were reported where such products caused fire when collected in a drum and where uninformed firemen increased the calamity when they tried to extinguish the fire with water. These are a few of the reasons that motivated tobacco producing companies organized in the CORESTA subgroup on pest control to look for new and less toxic ways of pest control.

One of the alternatives studied in this context was the use of Controlled Atmospheres. The efficacy of various CAs has been proven with many stored product insects at different temperatures, moisture contents and pressures (Lindgren and Vincent 1970, Jay et al. 1971, 1984a, 1984b, Bailey and Banks 1975, Annis 1987, Banks and Annis 1990, Navarro, Ripp et al. 1990, Adler 1994, Adler et al. 2000). The purpose of practical trials carried out at various locations was to prove the practical application of CAs in large-scale trials. The advantage of tobacco in this context is the high value per ton and the few potential pest species, namely the tobacco beetle *Lasioderma serricorne* (Col., Anobiidae) and the tobacco moth *Ephestia elutella* (Lep., Pyralidae). This paper describes results obtained with the tobacco beetle in trials carried out from 2009 to 2012. The main questions to be answered were:

1. Are the chosen conditions suitable for complete control of all stages of the tobacco beetle?
2. What is the optimum treatment temperature to secure a fast but still economically feasible pest control?
3. Are the chosen conditions achievable under practical conditions in a large storage chamber with some 100 so-called C48 cases of tobacco made of cardboard (weight 200 kg each)?

MATERIALS AND METHODS

Insect culture

Insects were reared at 25±1°C and 65±5% r.h. on wheat bran and broken tobacco leaves. The insect strain used was a phosphine resistant strain (COR 49) received from the Food and Environment Research Agency (FERA) in Sand Hutton, UK, or a phosphine resistant strain cultivated from a wild strain collected in Uganda. In the first trials 200 young beetles were placed onto 150 ml wheat bran (with glucose, glycerine and brewer's yeast added) and approx. 75 ml of broken tobacco. After 7 d the beetles were removed from the substrate and another 350 ml of wheat bran was added to provide additional feed to the developing larvae. This method was repeated in a weekly rhythm to receive the various developmental stages. In latter trials, 200 young beetles were placed onto cocoa powder. After 3 d the beetles were removed from the powder and batches of 80 eggs were counted into film tube cages together with 25 ml of wheat bran as substrate. This procedure was repeated weekly to receive the various developmental stages and gave more reliable numbers of individuals allowing calculation of percent mortality in case of survivors.

Preparation of samples and shipment
Eight developmental stages were taken from weekly cultures of the tobacco beetle. 50 adults were given into film capsules (length: 50 mm, diameter: 30 mm) together with 25 ml of fresh uninfested substrate. To allow easy gas exchange with the surrounding atmosphere, bottom and lid of the film capsules had an opening (diameter 6-10 mm) that was covered with a fine wire mesh gauze (mesh width approx 100 µm). Of eggs, larval and pupal stages, aliquots of 25 ml were taken from the respective culture jars. Four linen bags were filled with the eight film capsules, a fifth set of samples was kept as untreated control in a glass jar under laboratory conditions at 25°C. Data loggers to determine temperature and r.h. were added to the bags to be sent to Antwerp. Each bag was closed with a Velcro-fastener. In addition it was locked tightly with a metal wire. The four bags were placed into a cardboard box and were sent to Antwerp by mail on November 9, 2009. One of the four bags was an untreated control to determine the conditions during shipment, three bags were placed into tobacco bales at different positions within the treatment chamber. After treatment all four bags were sent back, opened, and checked for insect survival and mortality.

Treatment
In the majority of cases the treatment chamber consisted of a gastight cube with each side 10 m in length that was located inside an unheated storage building in Antwerp. It had a Salco door with a gas-tight seal and six 3000 W heating elements, as well as three fans placed at the sealing with an angle of 45%. The chamber was equipped with a nitrogen generator (membrane system) emitting nitrogen with a residual oxygen content of 0.5 %.
Prior to treatment the bags containing tobacco beetle stages and data loggers were placed into the tobacco in the C48 cases (Fig. 1).

Fig. 1- Insect sample bag in tobacco box prior to treatment

Handling after treatment

After treatment, samples were received back by mail and checked for survivors for at least 12 weeks. This was done because sub-lethal damages caused by CA are known to considerably delay developmental time (Adler and Reichmuth 1988). Climate data from shipment and treatment were turned into graphs and compared to figures given by operators responsible for the treatment. The hatch in shipped untreated controls was compared with hatch in untreated controls that had remained under laboratory conditions to determine the influence of adverse conditions during shipment on the results.

A total of 16 treatments were carried out in four different countries to test anoxic CAs with 0.5% O_2 at various temperatures and exposure times.

RESULTS

Heating large amounts of tobacco always resulted in a marked increase in relative humidity (Fig. 2). In Antwerp harbour, this lead to massive condensation at cool chamber walls during winter conditions.

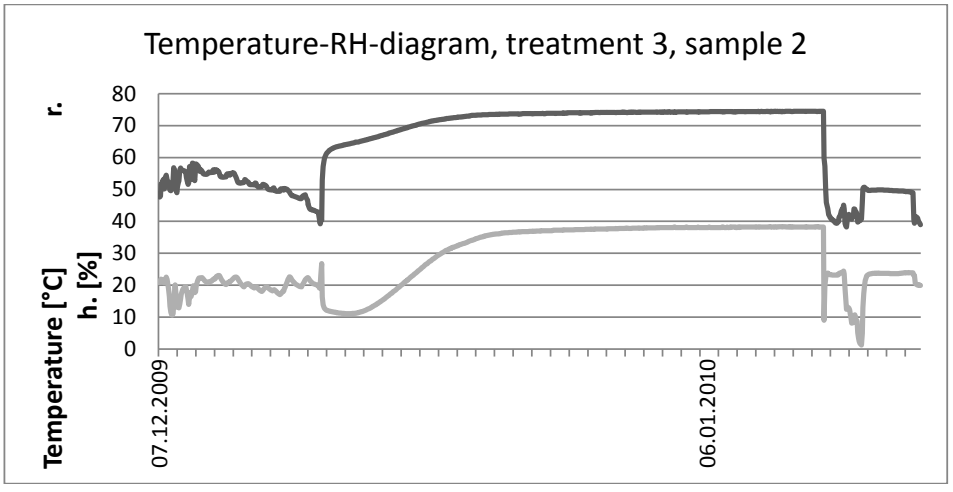

Fig. 2- Temperatures and r.h. over time in a treated sample during shipment and treatment. As may be noticed, humidity increased by approx. 20% during heating period and temperatures during shipment reached critically low levels on their way back to the laboratory.

During the first trials, temperature data of data loggers showed consistently much higher temperatures than those measured by the thermometers in the treatment chamber. Investigation showed that the temperature sensors in the chamber had not been calibrated.

During harsh frost periods in winter, shipment of samples could result in reduced numbers of survivors in the untreated control sent along with the samples compared to those of the sample remaining in the laboratory. From spring to early winter, however, hatch in both untreated samples was usually quite similar.

In a treatment in Indonesia were 38°C and a treatment time of 2.5 d at 0.5% O_2 had been chosen, few insects survived among old larvae of the tobacco beetles in our samples. At temperatures of 30°C or higher treatment times of 9 d were sufficient for complete control. Also at the same residual oxigen content, temperatures of 29°C, between 29 and 32°C and

higher temperatures for 9 d gave complete control. No survivors were found when 27°C were tested for 14 d, 25°C for 21 d, and 24°C for 28 d.

DISCUSSION

It could be seen that in heating stacked boxes or bales of tobacco an even distribution of temperatures is far from trivial. In many cases temperature recordings by data loggers differed by 2-5°C which could be attributed to their position in the stack. It is important to install strong transverse air currents directing hot air from the sealing down to the floor of a treatment chamber and to secure a good horizontal air flow, as well. The stacks of tobacco probably impede the air circulation and it could be useful to keep some free air space between the floor and the lowest tobacco box.

Fitness of a first strain utilized in this study seemed to be less than satisfactory when judged by numbers of offspring in untreated controls that had remained in the laboratory. Close investigation showed an infestation with microsporidia which motivated us to replace this strain by another phosphine resistant strain. Results obtained with the latter strain gave higher numbers of offspring but did not differ regarding the lethal effects caused by the CA treatment in combination with elevated temperatures.

The treatment at 38°C and 0.5% O_2 for 2.5 d seems to be a critical combination showing the first survivors in grown larvae close to pupation. From *Sitophilus granarius* it is known that this phase in juvenile development is most tolerant to CA treatments (Adler et al. 2000). The rice weevil *Sitophilus oryzae* could be controlled by CA at 38°C within 48 h (Jay 1987) and the granary weevil *S. granarius* at 40°C within 36 h (Adler 1997). However, the tobacco beetle was found to be comparatively tolerant to heat alone (Adler 2003). Obviously, the tobacco beetle is comparatively as tolerant as or even slightly more tolerant than the granary weevil to the combination of high temperatures and low levels of oxygen. A circumstance that may favour insect survival is the high relative humidity resulting from heating tobacco. It is known that part of the toxicity of anoxic atmospheres comes from dissiccation, and an increase in relative humidity may reduce the efficacy of this treatment.

It can be concluded that it is possible to achieve complete control of all stages of the tobacco beetle under the practical treatment conditions in a gastight chamber with a volume of approx. 1000 m^3. For the tobacco industry, a target residual oxygen content of 0.5%, temperatures of approx. 28°C, and a treatment time of some 9 d appear feasible also under economical aspects. Preheating cold tobacco may prolong total treatment times by several days. If the gastight chamber is in cold environment insulation could help to reduce the risk of condensation.

ACKNOWLEDGEMENTS

The authors are endebted to Debbie Collins from FERA, UK, for providing a strain of phosphine resistant tobacco beetles. They also thank Julia Dehmel for taking care of the insect cultures and technical assistance during the tests. We also want to extend our thanks to all members and companies enrolled in the CORESTA group.

REFERENCES

Adler C (1994) A comparison of the efficacy of CO_2-rich and N_2-rich atmospheres against the granary weevil *Sitophilus granarius.* Proceedings of the 6th International Working Conference on stored Product Protection, Canberra, April 1994: 11-15.

Adler C (1997) Rapid disinfestation through the combination of controlled atmospheres and heat. Donahaye, E.J., Navarro, S. and Varnava, A. (Eds.) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21-26 April 1996, Printco Ltd., Nicosia, Cyprus, 89-94.

Adler C (2003) Efficacy of heat treatments against the tobacco beetle *Lasioderma serricorne* F. (Col., Anobiidae) and the lesser grain borer *Rhyzopertha dominica* F. (Col., Bostrychidae). Advances in Stored Product Protection, Proceedings of the 8th International Working Conference on Stored Product Protection, York 2002, CAB International Publishing: 617-621.

Adler C, Reichmuth Ch (1988) Der Kornkäfer *Sitophilus granarius* (L.), Coleoptera, Curculionidae, seine Biologie und seine Bekämpfung in Getreide, insbesondere mit modifizierten Atmosphären. Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem, 239: 100pp.

Adler C, Corinth HG, Reichmuth Ch (2000) Chapter 5 - Modified atmospheres. In: Bh. Subramanyam & D. Hagstrum (eds.) Alternatives to pesticides in stored product IPM, Kluwer Academic Publ. Group, 105-146.

Annis PS (1987) Towards rational controlled atmosphere dosage scedules; - a review of the current knowledge. - In: Proc. 4th Int. Work. Conf. Stored-prod. Prot., Sept. 1986, Tel Aviv,Israel, Maor-Wallach Press: 128-148.

Bailey SW, Banks HJ (1975) The use of controlled atmospheres for storage of grain. - In: Anon.: Proc. 1st International Working Conference on Stored Product Entomology, Savannah, Georgia: 362-374.

Banks J, Annis PC (1990) Comparative advantages of high CO_2 and low O_2 types of controlled atmospheres for grain storage. - In: Calderon, M. and Barkai-Golan, R. (Eds.): Food Preservation by Modified Atmospheres, CRC Press, Boca Raton, Fla., USA 1990: 93-122.

Collins PJ, Emery RN, Walbank BE (2003) Two decades of monitoring and managing phosphine resistance in Australia. In: P.F. Credland, D.M. Armitage, C.H. Bell, P.M. Cogan and E. Highley: Advantages in Stored Product Protection, Proc. 8th Intern. Working Conf. Stored Product Protection, CABI Publishing, York 2002, 570-575.

Jay E, Arbogast RT, Pearman GC (1971) Relative humidity: its importance in the control of stored-product insects with modified atmospheric gas concentrations. - J. stored Prod. Res. 6: 325-329.

Jay, E. 1984a: Recent advances in the use of modified atmospheres for the control of stored-product insects. - In: F. Baur (Ed.): Insect Management for Food Storage and Processing. Am. Ass. Cereal Chemists, Inc., St. Paul, Minn.: 241-254.

Jay E (1984b) Imperfections in our current knowledge of insect biology as related to their response to controlled atmospheres. - In: Ripp, B.E., Banks, H.J., Calverley, D.J., Jay, E.G. and Navarro, S. (Eds.) (1984): Controlled Atmosphere and Fumigation in Grain Storages, Elsevier, Amsterdam: 493-508.

Jay E (1987) Factors affecting the use of carbon dioxide for treating raw and processed agricultural products. - In: GASGA Seminar on Fumigation Technology in Developing Countries, Slough, March 1986: 173-189.

Lindgren DL, Vincent LE (1970) Effect of atmospheric gases alone or in combination on the mortality of granary and rice weevils. - J. Econ. Entomol. 6: 1926-1929

Nayak M, Holloway J, Pavic H, Head M, Reid R, Patrick C (2010) Developing strategies to manage highly phosphine resitant populations of flat grain beetles in large bulk storages in Australia. In: Carvalho, O.M, Fields, P.G., Adler, C.S., Arthur, F.H., Athanassiou,

C.G., Campbell, J.F., Fleurat-Lessard, F., Flinn, P.W., Hodges, R.J., Isikber, A.A. Navarro, S., Noyes, R.T., Riudavets, J., Sinha, K.K., Thorpe, G.R., Timlick, B.H., Trematerra, P., White, N.D.G. (eds. 2010), Proceedings of the 10th International Working Conference on Stored Product Protection, 27 June-2 July 2010, Estoril, Portugal, Julius-Kuhn-Archiv 425, Berlin, Germany, 396-401.

Ripp BE, Largie de TA, Barry CB (1990) Advances in the practical application of controlled atmospheres for the preservation of grain in Australia. - In: Calderon, M. and Barkai-Golan, R. (Eds.): Food Preservation by Modified Atmospheres, CRC Press, Boca Raton, Fla., USA 1990: 151-184.