USING ENVIRONMENTALLY FRIENDLY ALTERNATIVES TO CONTROL POST-HARVEST INSECT PESTS

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In grain production, bringing in the harvest does not at all end the possibility of crop losses caused by herbivorous insects. Each year, storage insect pests cause significant losses in stored products worldwide. Use of modified atmospheres with depleted O_2 (hypoxia) is an environmentally friendly alternative to currently used chemical fumigation for control of storage insect pests. However, many insect species can adapt to O_2 deprivation, and recover from hours to days of hypoxia. This poses a difficulty in using the modified atmosphere technique. We systematically examined effects of low O_2 on cowpea bruchid development, profiled transcriptomic responses to hypoxia, and studied HIF1-regulated hypoxia-induced genes. Recent results on application of electron beam technology will also be presented here.

The cowpea bruchid, *Callosobruchus maculatus*, is an important storage pest of cowpea (*Vigna unguiculata*) and other legumes. Infestation of cowpea by bruchids starts in the field and proliferates in storage. The females lay their eggs on the surface of legume seeds, and larvae feed and develop inside the seeds. Adults start to lay eggs soon after they emerge, initiating another round of infestation. A few months of storage can result in 100% grain loss in unprotected cowpea seeds, particularly devastating for smallholding farmers.

First, we examined the impact of hypoxia and hypercapnia (high CO_2) on cowpea bruchids. Two O_2/CO_2 combinations were used; (a) 10% O_2 + 10% CO_2, (b) 2% O_2 + 18% CO_2. In both cases, N_2 was maintained at 80%, equivalent to normal atmospheric concentration. When exposed to 10% O_2 + 10% CO_2, eggs, larvae and pupae were able to complete their growth and successfully enter the next developmental stage, although developmental time and mortality varied at different stages. However, under more severe hypoxic/hypercapnic conditions, i.e. 2% O_2 + 18% CO_2, development of all stages ceased. Effects on eggs and adults were most dramatic, as they could only withstand 2-3 days of exposure. The 3rd and 4th instar larvae were least sensitive and could survive up to 20 days treatment. It should be noted that in ambient atmosphere, the rate of O_2 consumption and CO_2 output are greatest in the 3rd and 4th instar larvae compared to other stages. Results suggest that cowpea bruchids are able to tolerate a certain level of O_2 deprivation. Perhaps living in confined grains in storage, the bruchids could encounter hypoxic conditions. Insects that grow in such environments may have evolved an adaptive strategy to handle and survive a range of suboptimal O_2 concentrations.

In addition, to gain some molecular insight into the hypoxic/hypercarpnic response, we performed qPCR reactions on selected metabolic genes involved in the TCA cycle and in protein digestion, as well as genes encoding stress-responsive heat shock proteins. Patterns of gene expression and proteolysis suggest that cowpea bruchids suppress their metabolic activity and increase stress tolerance when challenged by O_2 deprivation. Transcript abundance as well as proteolytic activity recovered once normoxic conditions resumed. Taken together, cowpea bruchids were found able to cope with hypoxic and hypercapnic stress. This ability was particularly strong in the late larval stage.

Furthermore, we investigated the role of HIF1 in regulating transcript expression of representative heat shock proteins (HSPs) by promoter analyses. *HSP*s were induced by hypoxia treatment. Promoter analyses of representative *HSP* genes suggested that HIF1 positively regulates these genes. Its transcription activation function has been confirmed by transient co-transfection into S2 cells of constructs of HIF1 subunits and the *HSP* promoter-driven reporter.

Finally, we investigated electron beam (eBeam) sensitivity of cowpea bruchids at various developmental stages. Generated from linear accelerators, eBeam irradiation creates ions and radicals that are damaging to macromolecules such as DNA. It is safe, easy to operate, cost effective, and has a shorter treatment time than other irradiation methods. Moreover, there is no risk of potentially harmful residues remaining on the products. This irradiation technology has demonstrated great promise to disinfest a variety of stored products. We show that eBeam halted development of all developmental stages of cowpea bruchids, as well as reproduction of adult females.

In summary, better understanding of effects of the alternative pest management measures on target insects, as well as molecular mechanisms of the remarkable plasticity of the insects will help the design of strategies that are more effective against post-harvest insect pests. Knowledge obtained from cowpea bruchids can be extended to other economically important insect pests and will provide useful information for USDA-APHIS.