



Preservation of cowpea grain in sub-Saharan Africa—Bean/Cowpea CRSP contributions

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Abstract

In sub-Saharan Africa, post-harvest insect pests of cowpeas (*Vigna unguiculata* (L.) Walp.) degrade the nutritional quality and economic value of the grain and cause producers, in anticipation of losses during storage, to sell at harvest when the price is lowest. Principal pest is the cowpea bruchid, *Callosobruchus maculatus* (F.), but other bruchids cause losses as well. Beginning in the 1980s, the USAID-funded Bean/Cowpea Collaborative Research Support Program (CRSP) targeted post-harvest insect pests of cowpea as a constraint meriting an investment in research and development.

Subsequently, researchers in Senegal, Cameroon, and at Purdue University, created and helped disseminate numerous simple, low cost, and environmentally friendly technologies for managing post-harvest insect pests.

Technologies developed and disseminated with the help of NGOs such as World Vision International, the International Institute of Tropical Agriculture's PRONAF program, and FAO's Harare, Zimbabwe, office included: (1) a highly effective drum storage technology developed at ISRA, Senegal, and now widely adopted in Senegal; (2) a solar disinfestation technique developed at Purdue and at IRAD, Maroua, Cameroon, now being disseminated in many African countries; (3) an improved ash storage procedure; (4) a bagging technology utilizing triple plastic bags; (5) two cowpea cultivars expressing combined seed and pod wall resistance to cowpea bruchids, released by the Cameroon government in 1999.

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1. Introduction

Typically in sub-Saharan Africa, cowpea pods are hand-picked when mature, bagged, then hauled to a place where they are stored for a variable period until threshed. The grain is then stored for an additional

period, consumed, or sold. Initially shielded from insects within the harvested pods, the grain becomes more exposed to post-harvest insect pests after threshing, and is vulnerable to these insects throughout subsequent storage. Principal among these pests are the bruchids, or seed beetles, family Bruchidae. Singh and Jackai (1985) note that on-farm storage of cowpeas for 6 months is accompanied by about 30% loss in seed weight, with about 70% of the seeds being damaged and virtually unfit for consumption.

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Recent marketing studies have revealed that purchasers of cowpeas apply a discount from the first bruchid hole observed in a sample of grain for sale (Langyintuo et al., 2003).

The most important cowpea bruchid in sub-Saharan West Africa, *Callosobruchus maculatus* (F.) is often referred to as the cowpea “weevil”. The life history of the cowpea bruchid consists of egg, four larval instars, pupa and adult stages (Giga, 2001). The status of the cowpea bruchid as a pest owes to three life history traits; its high reproductive capacity, short developmental time, and continuous generations. An individual cowpea weevil female can reproduce herself 20–40-fold, and she is ready to mate and lay eggs immediately after emerging from the seed in which she developed. Egg hatchlings can produce reproductively active adults in as little as 3 weeks if temperatures are favorable. When a gravid female finds herself in a granary full of newly threshed seeds the stage is set for potentially disastrous losses. For example, if the initial infestation rate in a store is one gravid female per kilogram of grain, then after three generations have passed (less than 3 months) the damage level in the seeds can be greater than five holes per seed (R.E. Shade, unpublished observation). *Callosobruchus chinensis* L., the azuki bean weevil, has a similar life history, but is of more limited distribution than *C. maculatus*. In southern Africa is found *Callosobruchus rhodesianus* Pic, often together with the other two *Callosobruchus* species (Olubayo and Port, 1997; Giga and Smith, 1991). Another common bruchid in sub-Saharan Africa is *Bruchidius atrolineatus* Pic. Unlike *Callosobruchus* spp., *Bruchidius* does not reproduce continuously in storage. The females lay their eggs on the maturing cowpea pods in the field, and adults often emerge from the seeds during post-harvest storage. The infestation then dies out. *Bruchidius* appears to a serious problem only in some years, presumably when environmental conditions and other controlling factors such as parasitoids (Ndoutoume et al., 2000) favor high levels of field infestation (L. Murdock, unpublished observation).

In the autumn of 1986, the Bean/Cowpea CRSP Technical Committee determined that post-harvest losses of cowpea grain to insect pests were a serious constraint to the availability of cowpeas as food in West Africa, and a project design team was sent to Cameroon to determine if there was the basis there for

a project focused primarily on reducing post-harvest losses of cowpea grain. After nearly 2 weeks traveling in the north of Cameroon, the primary cowpea growing area, the team determined that there was indeed interest in cowpea storage problems within the Institut de la Recherche Agronomique (IRA), and that the facilities and trained personnel available in Maroua were adequate for development of such a project. The project was initiated in March, 1987, with Larry Murdock of Purdue University as US Principal Investigator, and Moffi Ta’Ama as Host Country Principal Investigator.

Cameroon is a country with much cultural and economic diversity. In northern Cameroon, where more than a score of different languages are spoken, cowpeas are grown by both men and women farmers, and for both home consumption and sale. On many farms the crop is grown in mixed culture, but some farmers also grow it as a monocrop. Surveys initiated in 1987 revealed that most farmers were aware of the bruchid problem, and often sold their newly harvested grain within a month or two of harvest, or consumed it quickly, thereby avoiding the worst of the post-harvest losses. The disadvantage of this was that they sold when the price was near its annual low point, but subsequently had to buy cowpeas on the open market, when the price was higher. While some farmers opted for the “sell or eat” strategy because of the bruchids; others made attempts to prevent bruchid infestations. These included keeping their cowpeas in pod form for 2 or more months on pole stands called dankis; putting the threshed grain in closed containers such as gourds or clay jars; mixing the grain with various amounts of ash from cooking fires; treating the grain with sundry insecticides (any available), or storing the grain with local herbs (Wolfson, 1990; cf. also Golob and Webley, 1980). Given this diversity, it was recognized that no single technology was likely to solve the bruchid problem. Accordingly, the project instead adopted a “smorgasbord” strategy, under which a variety of control technologies would be developed, with farmers and consumers themselves to choose from among the available alternatives the storage technology which best suited their needs. A basic assumption was that all technologies developed needed to be low cost, simple to use, and make use of materials readily available locally. One key value was that the technologies should be acceptable by potential users.

The latter point was assured by building technologies around observed farmer practices, and by involving farmers in the ongoing development and improvement of the technologies. Purdue took the lead in the more basic aspects of this research and in the early stages of development of candidate technologies, while IRA emphasized improving and adapting candidate technologies and working with farmers and in villages. The fundamental idea of the project was one of a partnership in which each of the principal parties did the things it could do best.

In addition to the new initiative in Cameroon, the Bean/Cowpea CRSP, with A.E. Hall as US Principal Investigator, and Dr. Mbaye N'Doye as Host Country Principal Investigator, had by the late 1980s already made strides to address the cowpea storage problem in Senegal. There an effective drum storage technique has been developed, as described below.

2. Drum storage

The technique developed by Dr. Dogo Seck and others (Seck and Gaspar, 1992) involves storing the cowpea grain in sealed metal drums. Sixty liter drums whose tops are fitted with 6–7 cm diameter screw-type plastic lids are filled to the top with dry threshed grain. Each drum holds about 45–55 kg, depending upon seed size. The filled container is sealed, with peanut or other cooking oil used to lubricate the edges of the closure to ensure an airtight seal. The oil also makes it easier to remove the lid after months of storage. The filled drums can be stored for 6 months with minimal losses to cowpea bruchids (Seck and Gaspar, 1992). The protective action during drum storage is likely due to depletion of oxygen and elevation of carbon dioxide concentration (Seck et al., 1996) that results from respiration of insects living in the grain at the time of storage, and to respiration of the grain itself. The drum technology was tested extensively over several years in northern and central zones of Senegal. It has since been adopted by about 80% of cowpea growers in Senegal (Faye and Lowenberg-DeBoer, 1999), an extraordinary rate of adoption that is evidence of the acceptability of the technology as well as of the recognition by farmers of the damage that bruchids can cause.

Drum storage, like all technologies, has limitations as well as advantages. Good quality used metal drums

are relatively expensive in much of Africa and hard to find in many places; this may limit the spread of the drum storage technology. In Senegal, by contrast, good metal drums are easily available and relatively inexpensive. The low rainfall and humidity in central and northern Senegal during much of the year, limits rust and prolongs the useful lives of drums to 10 years or more, making their use more economical. New drums are preferred because they do not have leaky seams or holes that sometimes are found in used or older drums. Air leaks lower the value of drum storage. Farmers have to be encouraged not to open the drums too soon after initiating the storage, for this admits air and allows surviving insects to resume feeding and development.

3. Improved ash storage

In many parts of sub-Saharan Africa, farmers often mix their cowpea grain with sieved ash from cooking fires, or with sand, in the hope of protecting their grain from bruchids (Golob and Webley, 1980). Surveys of cowpea storage by CRSP scientists in northern Cameroon confirmed that ash usage is common there, but farmers differed widely in the way they used ash, especially in the proportions of ash to grain. Some dusted their cowpeas lightly with ash, others used a large excess of ash over the grain, while still others used alternate layers of cowpeas and ash (Wolfson et al., 1991).

Given uncertainty about the effectiveness of ash as a grain protectant, systematic experiments were carried out at Purdue to determine whether ash was actually protective and to optimize the proportions of ash to grain required for protection. It was found that ash can indeed protect cowpea grain from runaway losses to cowpea weevil, but with some restrictions (Wolfson et al., 1991). One caveat is that any cowpea seed that already has a cowpea bruchid larvae developing inside it at the time the seed is mixed with ash will eventually have an emergence window, or even a hole. In short, ash does not prevent larvae already in the seed from completing their development. For this reason, grain not visibly infested by cowpea weevils (no adult exit holes) can be put into ash storage and yet show emergence holes when the store is opened weeks or months later. Some women who store cowpeas in ash

had observed this, and as a result had doubts about the effectiveness of ash.

The proportions of ash to cowpea grain needed for complete protection are three (or more) volumes of ash to four volumes of cowpea grain—mixed thoroughly and packed firmly into a container such as a calabash or a clay jar, with a 3 cm layer of ash placed on top. When these proportions are used, bruchid population growth is completely arrested, and no eggs appear on the grain. While the ash may act in part by its abrasive action on the insect cuticle, causing desiccation and death of the insect, it probably acts primarily by entrapment. Adults that develop within the grains are simply physically unable to shove their way out of the seeds, and so are entombed, and die without mating. One of the observations made during these experiments is that a cache of uninfested cowpeas covered with a 3 cm layer of ash would never become infested even if it were exposed to large numbers of bruchids, because adult bruchids will never dig downwards to reach the grain.

Based on these results and on extensive field testing in Cameroon, CRSP scientists recommend the following procedure for using ash to store cowpea grain. Mix equal volumes of sieved ash and cowpea grain, place the mixture in a container, and cover the ash/grain mixture with a 3 cm layer of ash. Grain stored in this way can be kept for long periods of time with minimal losses. A technical bulletin (Kitch and Ntougam, 1991a) describing the procedure has been published and widely disseminated. Advantages of the ash storage technique are its simplicity and very low cost. The major disadvantage is that it is suitable for only small volumes of grain. When larger amounts of grain need to be stored, the volume of ash and the space required become prohibitive.

Not all Cameroon farmers find the ash storage technique acceptable. During a demonstration in a village on the Chad border, several CRSP storage technologies (see below) were set up for people to view. More than 100 villagers came to the demonstrations, but it became apparent in discussions that the farmers were not interested in the ash treatment. When asked why, a villager explained that when twins were born to a family—it was considered a sign of bad luck and ash from the wood of a certain tree was put in the mouth of one of the children, causing it to die. Ash and death were too closely associated for the people in that

particular village for them to even think of using ash in association with food.

4. Solar disinfection

African farmers often spread their harvested grain on mats or at the edge of the road or on flat stones, leaving it in the sun to dry. This custom, together with the fact that sunlight is abundant in northern Cameroon once the rains end in the autumn, led to a novel approach to controlling cowpea bruchids. All insects have thermal death points, a temperature at which they are unable to survive. In the case of the cowpea bruchid this is 57 °C, with all life stages of the insect (egg, larvae, pupa and adult) killed when exposed to this temperature for 1 h (Murdock and Shade, 1991).

To achieve this temperature, and thus disinfect cowpeas, Murdock and Shade used plastic sheeting to enclose and heat the cowpea grain. Black plastic sheeting (woven wicker mats can serve nearly as well) is laid upon the ground, and then covered to a depth of 1–2 cm with infested cowpea grain. A second, translucent plastic sheet is used to cover the lower sheet and grain, then the edges of the two plastic sheets are sealed by folding the upper sheet under the lower one and securing the envelope so formed with small stones laid around the edges. When exposed to the sunlight, the temperature within the envelope rises rapidly thanks to solar energy passing through the translucent upper sheet and being absorbed by the cowpea grain and the underlying black plastic sheet. Within 15–30 min the temperature within the cowpea grain typically rises to 60–70 °C, more than adequate to kill all stages of the cowpea weevil (Murdock and Shade, 1991).

After this prototype heater was developed at Purdue, it was adapted and improved for use by low resource farmers in Cameroon. Two major improvements were the addition of an insulating layer placed between the ground (typically bare sand) and the undersurface of the black plastic sheeting. Dry grass pulled from the roadside provided needed insulation. Without this, it was more difficult to attain within-seed killing temperatures, probably because heat was otherwise rapidly lost to the underlying earth by conduction. The second major improvement was expansion of the heater size to 3 m × 3 m. This enabled the heater to

be used with 50–60 kg of grain at a time. After trials in villages in northern Cameroon, it was determined that the heater was practical, useful, and economical (Kitch et al., 1992).

One of the favorable features of solar disinfection is that solar-treated cowpea grain can be used in any way that untreated grain can be. Solar disinfection, despite the high temperatures to which the grain is exposed, germinates and cooks normally. In fact, cowpea grain could be heated to 80 °C for up to 6 h without any significant effect upon germination. We speculate that this enormous heat tolerance of cowpea owes to its evolution in the arid Sahel, where it naturally had to be able to withstand high temperatures to survive.

A technical bulletin describing the solar heater is available in French, English, and Fulfulde (Ntoukam and Kitch, 1991). Subsequent to release of details for the heater it was shown that numerous farmers in a village can share a heater and that individual heaters could be used for 2 years or more before they became too tattered to be effective. In the late 1990s, a survey of adoption revealed that about 10% of the farmers in northern Cameroon had adopted this or other solar heaters. Many technicians and associates from World Vision International, an NGO active in Africa, have been trained in the use of the solar heater. IITA, through its PEDUNE and PRONAF projects has also been active in disseminating this technology as well as the triple bagging technique described below. In 2000, FAO became interested in helping bring grain preservation technologies to the southern and eastern Africa region. A joint workshop involving the CRSP, FAO, and the Crop Post-Harvest Programme was organized. The published proceedings (Kitch and Sibanda, 2001) contain detailed descriptions of bruchid pests of cowpeas as well as numerous storage technologies.

Solar heating can also be used for cowpea germplasm preservation. As with other stored cowpeas, bruchids are a continual threat to seeds making up national germplasm collections in Africa. Fumigation is sometimes used for bruchid control, but is effective for only limited periods of time. In the absence of timely re-fumigation, important germplasm samples or breeding lines can be lost. When small ziplock plastic bags containing grain of particular cowpea accessions were placed in the 3 m × 3 m large solar

heater developed for on-farm disinfection of bulk grain, the heat generated within the heater killed all bruchids living within the seeds. Germination was not affected by the heat, and subsequent storage of the grain in the plastic ziplock bags prevented re-infestation (Ntoukam et al., 1997). Thus, the solar heater offers an alternative, simple, low cost technique for preserving cowpea germplasm collections without the use of fumigants.

5. Triple bagging

When the Bean/Cowpea CRSP storage project began in 1987, Dr. Moffi Ta'Ama had been experimenting with fumigants for the control of the cowpea bruchid. Plastic bags were widely used for grain storage in northern Cameroon, and so Ta'Ama attempted to use them as fumigation containers. In one experiment he filled several large, double plastic bags (one bag inside the other) with bruchid infested cowpea grain, then treated some bags with carbon tetrachloride, while others received no treatment as a positive control. All bags were tied securely shut and set off in a corner of the lab. When the bags were opened several months later both carbon tetrachloride-treated and untreated grain were free from bruchids.

Subsequent systematic studies by CRSP researchers confirmed that merely confining infested grain in multiple tightly closed plastic sacks, one enclosed within the other, is sufficient to arrest a cowpea bruchid infestation. On-farm tests with Cameroon villagers validated the effectiveness of this methodology, called "triple plastic bagging". The recommended procedure consists of filling a plastic bag with infested cowpea grain, tying the mouth of the bag shut, enclosing this bag completely within a second one, and tightly securing that, then repeating the procedure using a third bag. The third bag was added as an insurance measure. The method is simple, uses readily available materials, and is at low cost. The principle by which triple bagging works has not been studied, but the likely mechanism involves oxygen depletion and elevation of carbon dioxide levels, as occurs with sealed drum storage (Seck et al., 1996). Respiration of insects living in seeds stored in a closed space may, together with respiration of the grain itself, in combination with the limited free oxygen available,

eventually reduce the oxygen levels and elevate the carbon dioxide levels to a point where the insects are unable to carry out their life processes normally. The bagging procedure does not appear to kill the bruchids, since it has often been observed that live adult insects can be seen moving around in grain that has been stored in triple bags for several months. Bruchids that survive in the grain presumably are inactive, and resume activity only when oxygen again becomes available. A technical bulletin describing the procedure has been published (Kitch and Ntoukam, 1991b).

6. Biology and behavior of cowpea weevil—new insights

When insects feed within the hard tissues of seeds, wood, or other dry brittle biological material, they typically generate extremely weak but still detectable ultrasonic signals (Shade et al., 1989). With a piezoelectric transducer and appropriate electronic circuitry to filter and amplify the signals, and with an audio-amplifier and speakers, it is possible to listen to the

feeding activity of cowpea bruchid larvae living within cowpea seeds. Virtually every biting event—the larvae are observed to rear back within their tunnels and strike the surface of the seed material with their heads—can be heard, from the time the larvae hatches from the egg and bores into the seed until it emerges as an adult roughly 2 weeks later. This phenomenon has led to the creation of the Purdue Insect Feeding Monitor, which has given new insights into the life of insects feeding hidden in seeds and other dry materials. For example, when a larva develops in a susceptible cowpea (Fig. 1), feeding rates increase at each instar, and reach peak values in the fourth-instar (Shade et al., 1990). Complete cessation of feeding activity occurs at each molt. Continual monitoring throughout the larval life of the insects reveals a detailed picture of the life history of the insect. One of the great advantages of monitoring in the ultrasonic range is that, unlike acoustical monitoring, the ultrasonic background noise is practically nil. It is possible to work around the ultrasonic monitor, to talk and to carry out normal tasks without causing extraneous signals.

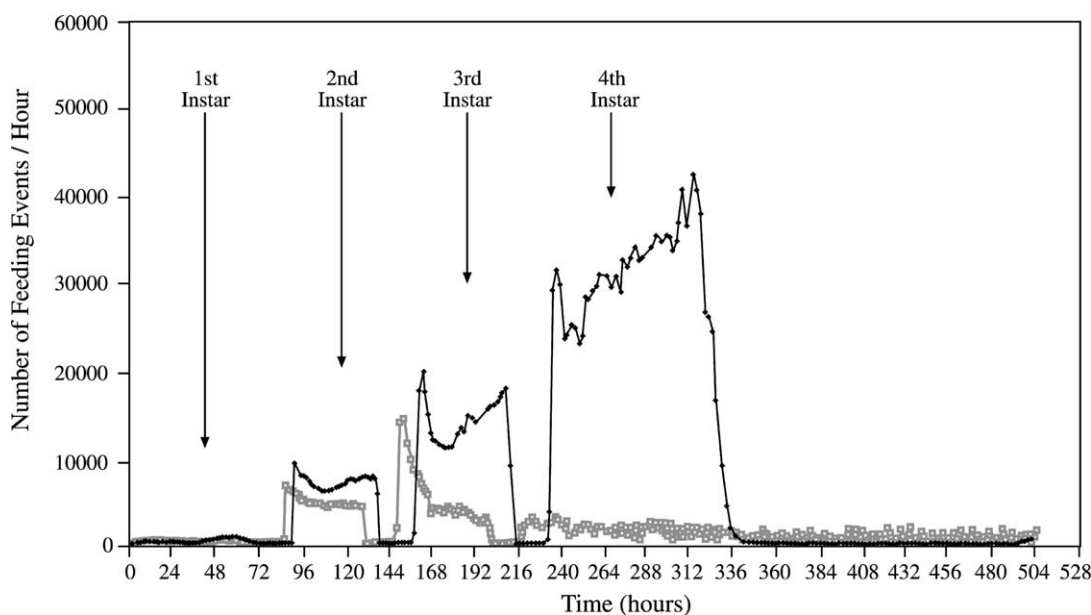


Fig. 1. Feeding activity throughout the entire within-seed life of two larval cowpea bruchids (*Callosobruchus maculatus*). One larva fed in a susceptible California Blackeye No. 5 seed (◆); the other fed in a resistant TVu 2027 seed (□). Feeding activity during the first instars of both insects was similar, but after the second molt (as reflected by cessation of all feeding), the feeding rate of the larva in the TVu 2027 seed fell rapidly and never recovered. Feeding was evident subsequently, but only at a low, irregular frequency. Arrows indicate each of the larval instars for the insect feeding in the susceptible seed.

7. Applications of the feeding monitor

Singh (1977) reported that seeds of cowpea land-race TVu 2027 are moderately resistant to the cowpea weevil, with developmental times markedly prolonged, and increased larval mortality. When the feeding activity of larvae living in TVu 2027 seeds was monitored, it was found that feeding rates and rates of development were essentially normal in the first two larval instars, but dropped markedly in the third-instar. Feeding during the third- and fourth-instars, if the larvae survived to this stage, was severely depressed, and the duration of the fourth-instar was greatly prolonged (Fig. 1). These marked differences in feeding behavior can be used to easily and rapidly screen large numbers of seeds from germ-plasm collections or breeding programs for differences in susceptibility to cowpea bruchid. One of the prerequisites for large-scale application of the feeding monitor is the availability of a multi-channel monitor. The Purdue group has built 16-channel monitors, but in principle there would be no difficulty in building them with 100-channels or more. Another prerequisite is the capability to manage the data generated during recording of feeding activity involving large numbers of bruchids and seeds. For this, the Purdue group has adapted the data-collection and management program LabView VI to collect, analyze and display the data from 16-channel monitors. Data management for the processing of large numbers of samples is non-trivial, so a system of labeling each sample with miniature bar-code labels has been devised which allows each seed to be tracked through the evaluation process.

Detailed comparisons of the life histories of cowpea bruchid larvae feeding in susceptible (California Blackeye No. 5) or resistant (TVu 2027) seeds led to the following protocol for screening germplasm accessions for resistance. Each seed is infested with a single egg and kept for 9 days post-egg hatch in the rearing room at 26 °C and 40–50% RH. On the 10th day, each infested seed is placed on a transducer of the multi-channel biomonitor, left to adapt for 5 min, and then monitored for five additional minutes. Blind experiments revealed that this single brief period of monitoring correctly identifies resistant seeds 95% of the time. Occasionally a susceptible seed will be incorrectly identified as resistant. This probably

occurs because an occasional normal larva feeding in a susceptible seed may be modestly retarded in its development, for whatever reason, and is still in the molting stage at the time monitoring occurs. This erroneous misidentification of a susceptible seed as resistant is easily corrected by re-monitoring seeds identified as resistant again on the following day. Any larva that was inactive because it was molting at the time of monitoring the day before will now be actively feeding and the seed in question will be correctly categorized.

With a 48-channel monitor, a single technician could easily monitor 144 seeds an hour or about 1000 seeds a day. This offers a simple, economic and rapid method for the evaluation of large germ-plasm holdings, and is in contrast to the previous screenings of the world germplasm collection that have relied on a bulk procedure that would have allowed individual highly resistant seeds to escape detection.

8. Potential of biotechnology for cowpea grain storage

One of the more remarkable characteristics of bruchid beetles is the degree of host plant specialization they can exhibit. Close association of a given bruchid species with seeds of a particular legume species has often been the basis for the common names of the insects. Thus, there is an adzuki bean weevil, a cowpea weevil, and a common bean weevil, among others. A given bruchid species may feed on seeds of one species and not on those of another, fairly closely related legume. An example is the cowpea bruchid, which thrives in cowpea seeds and develops in adzuki beans (*Vigna angularis* (Willd.) Ohwi and Ohashi) and garden pea seeds (*Pisum sativum* L.) but fails utterly to survive in seeds of common bean (*Phaseolus vulgaris* L.). Detailed studies of the basis of resistance of common beans to the cowpea bruchid (Ishimoto and Kitamura, 1989; Huesing et al., 1991) established that the cowpea bruchid fails to develop in common bean seeds because of the presence in the seeds of a proteinaceous alpha-amylase inhibitor.

This fact led to the hypothesis that transfer of the gene encoding the alpha-amylase inhibitor into cowpea would confer resistance to the bruchid.

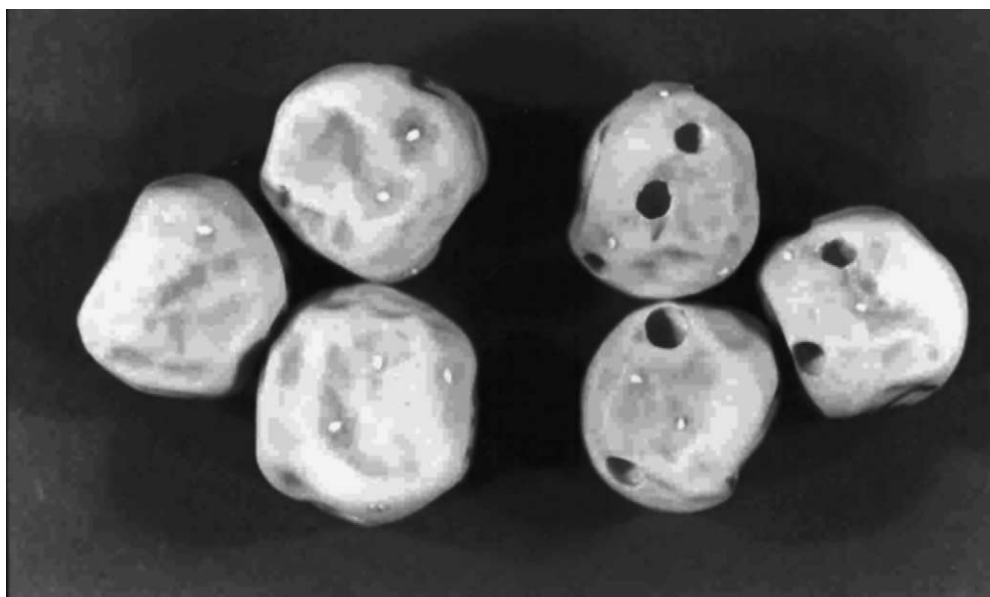


Fig. 2. Transgenic garden peas (*Pisum sativum*) expressing the alpha-amylase inhibitor from common bean (*Phaseolus vulgaris*) are highly resistant to cowpea bruchid (left side), while untransformed peas (right side) exhibit bruchid emergence holes.

Unfortunately it was not possible to test this hypothesis using cowpea itself (*Vigna unguiculata* (L.) Walp.) because there is currently no effective procedure for genetic transformation of cowpea. Instead garden pea, the seeds of which can serve as hosts for cowpea weevil larvae, and for which there is an efficient, reproducible transformation system (Schroeder et al., 1993) was used. Accordingly, a multidisciplinary team based at CSIRO in Australia, at the University of California at San Diego, and Purdue University developed transgenic garden peas expressing common bean alpha-amylase inhibitors in its seeds. The results were clear-cut and definitive (Fig. 2). Garden peas expressing common bean alpha-amylase inhibitor at the same levels as occur in common bean (about 1.0%, w/w) were immune to the cowpea bruchid (Shade et al., 1994). When cowpea weevil eggs were laid on these peas, the eggs hatched normally and the larvae bored into the seeds, but then subsequently died. The degree of protection was proportional to the amounts of alpha-amylase inhibitor expressed in the seeds.

When a reliable, efficient and reproducible methodology for genetic transformation of cowpea becomes available, we will be in a position to develop a novel source of resistance to the cowpea bruchid. It may be useful to point out that the gene being considered for

this purpose, alpha-amylase inhibitor, is already a common constituent of human and animal foods made from common bean, and that the protective gene product, alpha-amylase inhibitor, is degraded by heat, during cooking.

9. Biotypes of the cowpea weevil

One of the reasons for seeking new sources of resistance to the cowpea weevil is that there is only a single known natural source of resistance, namely the cowpea landrace TVu 2027 (Singh, 1977). Two other sources of resistance, accessions TVu 11952 (KNW) and TVu 11953 (KNS) were subsequently shown to owe their resistance to the same genes as TVu 2027 (Kitch, 1987). In each case, some as yet unknown factor in the seeds retards larval development, and causes increased larval mortality. Given the extremely limited number of genes available to breeders, it is likely that cowpea bruchid biotypes may develop the ability to overcome this resistance. Indeed, it appears that this has already happened. Over the years, the CRSP storage entomology team at Purdue collected and maintained 26 different populations of cowpea weevil from different localities in

West Africa. One of these populations, obtained from IITA, performed very well on TVu 2027 (Shade et al., 1996).

A cowpea bruchid population capable of overcoming the resistance of TVu 2027 was also established from selection experiments carried out at Purdue. A population of bruchids from Niger was reared on TVu 2027 seeds. Initially, they performed poorly on this host, with long developmental times (mean = 55 days) and high mortality (mean = 68%) compared to individuals feeding in susceptible seeds (California Blackeye No. 5; developmental time = 28.5 days, mortality 20–30%). The selection regime imposed on the population reared on TVu 2027 was as follows: the first 10–15% of individuals emerging from each generation were selected and allowed to mate, then exposed to a fresh population of TVu 2027 seeds to begin the next generation. This procedure was continued for 77 generations, and required 8 years of work. In essence, there was selection for the fastest developing individuals in each generation. Over the length of the experiment, the developmental time on TVu 2027 gradually decreased by a mean of 0.34 days per generation. After about 50 generations of selection, the selected population could develop on TVu 2027 about as fast as it could on susceptible seeds.

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References

- Faye, M., Lowenberg-DeBoer, J., 1999. Adoption of cowpea improved varieties and storage technology in the north central peanut basin of Senegal and economic impact implications. Bean/Cowpea CRSP, West Africa Social Science Working Group Report No. 3.
- Giga, D.P., 2001. Biology and ecology of *Callosobruchus* species. In: Kitch, L., Sibanda, T. (Eds.), Post-Harvest Storage Technologies for Cowpea (*Vigna unguiculata*) in Southern Africa. FAO Publication, Harare, Zimbabwe.
- Giga, D.P., Smith, R.H., 1991. Intraspecific competition in the bean weevils *Callosobruchus maculatus* and *Callosobruchus rhodesianus* (Coleoptera: Bruchidae). J. Appl. Ecol. 28, 918–929.
- Golob, P., Webley, D.J., 1980. The use of plants and minerals as traditional protectants of stored products. Tropical Products Institute Publication No. G138, Overseas Development Administration, London.
- Huesing, J.H., Shade, R.E., Chrispeels, M.J., Murdock, L.L., 1991. Alpha-amylase inhibitor, not phytohemagglutinin, explains resistance of common bean seeds to cowpea weevil. Plant Physiol. 96, 993–996.
- Ishimoto, M., Kitamura, K., 1989. Growth inhibitory effects of alpha-amylase inhibitor from the kidney bean, *Phaseolus vulgaris* (L.) on 3 species of bruchids (Coleoptera: Bruchidae). Appl. Ent. Zool. 24, 281–286.
- Kitch, L.W., 1987. Relationship of bruchid (*Callosobruchus maculatus*) resistance genes in three cowpea cultivars. Ph.D. Thesis. Department of Agronomy, Purdue University, West Lafayette, IN, USA.
- Kitch, L.W., Ntoukam, G., 1991a. Storage of cowpea in ash. Technical Bulletin No. 1. Institut de la Recherche Agronomique du Cameroun (IRA) and Bean/Cowpea Collaborative Research Support Program (CRSP) (Published in English, French, and Fulfulde).
- Kitch, L.W., Ntoukam, G., 1991b. Airtight storage of cowpea in triple plastic bags. Technical Bulletin No. 3. Institut de la Recherche Agronomique du Cameroun (IRA) and Bean/Cowpea Collaborative Research Support Program (CRSP) (Published in English, French, and Fulfulde).
- Kitch, L.W., Sibanda, T., 2001. Post-harvest storage technologies for cowpea (*Vigna unguiculata*) in Southern Africa. In: Proceedings of the Regional Workshop, Harare, Zimbabwe, 12–14 April, 2000. FAO Publication, Harare, Zimbabwe.
- Kitch, L.W., Ntoukam, G., Shade, R.E., Wolfson, J.L., Murdock, L.L., 1992. A solar heater for disinfesting stored cowpeas on subsistence farms. J. Stored Prod. Res. 28, 261–267.
- Langyintuo, A.S., Lowenberg-DeBoer, J., Faye, M., Lambert, D., Ibro, G., Moussa, B., Kergna, A., Kushwaha, S., Musa, S., Ntoukam, G., 2003. Cowpea supply and demand in West and Central Africa. Field Crops Res. 82, 215–231.
- Murdock, L.L., Shade, R.E., 1991. Eradication of cowpea weevil (Coleoptera: Bruchidae) in cowpeas by solar heating. Am. Entomol. 37, 228–231.
- Ndoutoume, A., Kalmes, R., Rojas-Rousse, D., 2000. Reproductive potential of *Eupelmus orientalis* (Crawford) and *Eupelmus villeti* (Crawford) (Hymenoptera: Eupelmidae), two parasitoids of Bruchidae (Coleoptera) during the harvest and storage of cowpea pods (*Vigna unguiculata* (L.) Walp.). African Entomol. 8, 201–209.
- Ntoukam, G., Kitch, L.W., 1991. Solar heaters for improved cowpea storage. Technical Bulletin No. 2. Institut de la Recherche Agronomique du Cameroun (IRA) and Bean/Cowpea Collaborative Research Support Program (CRSP) (Published in English, French, and Fulfulde).

- Ntoukam, G., Kitch, L.W., Shade, R.E., Murdock, L.L., 1997. A novel method for conserving cowpea germplasm and breeding stocks using solar disinfestation. *J. Stored Prod. Res.* 33, 175–179.
- Olubayo, F.M., Port, G.R., 1997. The efficacy of harvest time modification and intercropping as methods of reducing the field infestation of cowpeas by storage bruchids in Kenya. *J. Stored Prod. Res.* 33, 271–276.
- Schroeder, H.E., Schotz, A.H., Wardley-Richardson, T., Spencer, D., Higgins, T.J.V., 1993. Transformation and regeneration of two cultivars of pea (*Pisum sativum* L.). *Plant Physiol.* 101, 751–757.
- Seck, D., Gaspar, C., 1992. Efficacité du stockage du niébé (*Vigna unguiculata* (L.) Walp.) en fûts métalliques hermétiques comme méthode alternative de contrôle de *Callosobruchus maculatus* (F.) en Afrique sahélienne. *Med. Fac. Landbouww. Rijksuniv. Gent* 57 (3), 751–758.
- Seck, D., Haubruge, G., Marlier, E., Gaspar, C., 1996. Alternative protection of cowpea seeds against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) using hermetic storage alone or in combination with *Boscia senegalensis* (Pers) Lam ex Poir. *J. Stored Prod. Res.* 32, 39–44.
- Shade, R.E., Furgason, E.S., Murdock, L.L., 1989. Ultrasonic insect detector. US Patent 4 809 554. Assignee: Purdue Research Foundation, West Lafayette, IN.
- Shade, R.E., Furgason, E.S., Murdock, L.L., 1990. Detection of hidden insect infestations by feeding-generated ultrasonic signals. *Am. Entomol.*, 231–234.
- Shade, R.E., Schroeder, H.E., Pueyo, J.J., Tabe, L.M., Murdock, L.L., Higgins, T.J.V., Chrispeels, M.J., 1994. Transgenic pea seeds expressing the alpha-amylase inhibitor of the common bean are resistant to bruchid beetles. *Biotechnology* 12, 793–796.
- Shade, R.E., Kitch, L.W., Mentzer, P., Murdock, L.L., 1996. Selection of a cowpea weevil (Coleoptera: Bruchidae) biotype virulent to cowpea weevil resistant landrace TVu 2027. *J. Econ. Entomol.* 89, 1325–13331.
- Singh, S.R., 1977. Cowpea cultivars resistant to insect pests in world germplasm collection. *Trop. Grain Legume Bull.* 9, 1–7.
- Singh, S.R., Jackai, L.E.N., 1985. Insect pests of cowpeas in Africa: their life cycle, economic importance and potential for control. In: Singh, S.R., Rachie, K.O. (Eds.), *Cowpea Research, Production and Utilization*. Wiley, New York, pp. 217–232.
- Wolfson, J.L., 1990. Preservation of post harvest cowpeas by subsistence farmers in Cameroon. *Bean/Cowpea CRSP FY 90 Annual Report*. Michigan State University, East Lansing, MI, pp. 63–81.
- Wolfson, J.L., Shade, R.E., Mentzer, P.E., Murdock, L.L., 1991. Efficacy of ash for controlling infestations of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpeas. *J. Stored Prod. Res.* 27, 239–243.