POTENTIAL USES OF HOST PLANT RESISTANCE

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Introduction

The study of the resistance of crops to post harvest infestation by stored products insects is a subject that lends itself well to laboratory investigation. The insects are easy to rear, the experiments can be carried out using relatively small amounts of material and no complicated or expensive facilities are required. Unfortunately, investigations that are carried out to exploit the suitability of the laboratory environment usually remain laboratory based and may not lead to any practical advances towards the development of resistant crop varieties. This is unfortunate, as it is clear that there is a great opportunity to improve the resistance to insects of crops after harvest.

In the areas of the world where post harvest insect infestation is serious it is a common observation that farmers' traditional varieties are usually reasonably resistant to local pests, and that introduced varieties are often more susceptible (Rodriguez, 1976 and Fortier et al, 1982). It seems that natural selection combined with selection by the farmer ensures that local varieties remain resistant. Recent work at the Tropical Development and Research Institute (TDRI) has confirmed the relative resistance of varieties from areas where storage pests are serious. The Institut de Recherches Agronomiques Tropicales (France) provided a series of maize cultivars representing its extensive germplasm collection. The varieties were tested for their resistance to Sitophilus zeamais Motsch. (using the method of Dobie, 1974) and were then grouped according, as far as was known, to their geographical origin. The result is shown in Figure 1. Varieties originating from areas such as the Caribbean and Central America are relatively resistant. However the group that consists mainly of collections from sub-Sahelian regions of West Africa lack resistance and are generally very susceptible to Sitophilus attack. Sitophilus spp. are very uncommon in the arid regions of the Sahel, and no selection, either natural or artificial would have affected varieties from that region.

Varieties developed for the rural community, therefore, should be adequately resistant to storage pests, but how resistant is adequate ? In recent years a great deal of effort has been made towards objectively measuring the losses to grain caused by insects. It has become apparent that traditional varieties stored using traditional methods do not suffer heavy losses. In a study in Malawi, Golob (1981a, b) found that weight loss over 10 months storage was 3% or less for maize and less than 2% for sorghum. In a second survey in a different part of the country he recorded losses of less than 1.5% for maize and less than 1% for groundnuts. He noted that all the crops in

Proc 4th Int Work Conf Stored-Product Protection, Tel Aviv, Israel, Sept 1986
[Eds E Donahaye and S Navarro], pp 2-13

the study area were relatively resistant, and commented on the "considerable likelihood of losses with high-yielding but more susceptible varieties if these were introduced to the area". Thus for rural areas it will often be sufficient to ensure that the susceptibility of introduced varieties is no poorer than that of the varieties already grown and stored in the region. It may be unnecessary to attempt to introduce very high levels of resistance as the local storage methods may be perfectly adequate for the local crops. Thus plant breeding programmes aimed at small scale farmers in developing countries need have relatively modest objectives insofar as resistance to storage pests is concerned. In order to show how resistance to storage pests can be exploited, two case histories will be discussed.

The development of resistant crops: two case histories

Cowpeas

Cowpea (*Vigna unguiculata*) is the most important grain legume crop in West Africa. It is also widely grown in large areas of Asia, parts of South America and (as a commercial crop) in the U.S.A. and Australia.

In tropical areas the harvested seeds suffer heavy damage by bruchid beetles of the genus *Callosobruchus*. Unlike cereals, there appears to be little natural resistance to insect attack, and losses are often very high. In an attempt to discover a source of resistance to bruchid infestation, investigators at the International Institute of Tropical Agriculture (IITA) screened several thousand varieties from their international germplasm collection, and were initially successful in discovering only one variety (known as TVu 2027) that showed resistance to *Callosobruchus maculatus* (Singh, 1978). Fortunately, the level of resistance was very high, and action was taken to investigate and exploit the resistance. The aspect of the work that I shall describe was a collaborative effort involving IITA, the Botany Department of the University of Durham in the U.K. and entomologists at TDRI.

The preliminary work carried out in the U.K. was intended to confirm the high reported levels of resistance in samples of cowpea variety TVu2027 supplied by IITA, and bio-assays of the whole beans showed that they were highly resistant (Table 1). Our collaborators at the University of Durham then started to investigate the biochemistry of the resistant variety, with a view to identifying anti-metabolic compounds that may have caused the resistance. Many possible antimetabolites such as lectins and non-protein amino acids were eliminated, and it was quickly established that the resistant variety had an abnormally high level of a proteinase inhibitor. The research then took two paths. At TDRI a bio-assay was developed to allow the testing of the antimetabolic properties of the proteinase inhibitor. Purified inhibitor was introduced into a diet based on the ground cotyledon of a susceptible variety of cowpea and adult *C. maculatus* were induced to lay eggs on small samples of diet wrapped in a PVC film. The results of the bio-assay demonstrated that the inhibitor had a marked effect on the development of *C. maculatus* (Fig. 2)

(Gatehouse et al, 1979). Research at Durham then continued and it was demonstrated in vitro that the inhibitor from the resistant variety directly inhibited the proteinase enzyme from the gut of C. maculatus: thus the larvae feeding on the cotyledons of the resistant cowpeas would be unable to efficiently digest protein (Gatehouse and Boulter, 1983). It was also found that the inhibitor was unevely distributed in cowpea seeds, most of it being located in the cotyledon just below the testa. Thus the seed is very well protected against first instar bruchid larvae boring into the seed from outside, but it is difficult to relate overall concentrations of the inhibitor in the seed to its anti-metabolic effect as it is most concentrated in regions where the seed is most susceptible to attack. However, the studies also revealed a complication in the interpretation of the effect of the proteinase inhibitor: the inhibitor became ineffective in a diet that was supplemented by sulphur bearing amino acids. It was therefore likely that the expression of resistance in cowpea varieties would depend not only upon the presence of the inhibitor but also upon the quality of other proteins found in the cowpea cotyledon.

Meanwhile, in Nigeria, IITA started to investigate the inheritability of resistance. Crosses were made between TVu2027 and other agronomically superior cowpea lines, and seed of crosses and backcrosses was sent to TDRI and Durham for insect bio-assay and proteinase inhibitor analysis. It was found that inheritance of resistance was controlled by one or two major genes with modifiers (Redden et al, 1983; Redden, 1983). Selection of pure breeding resistant lines proceeded for several generations, but segregation for selection continued to be a problem for breeders for several generations (as would be expected knowing that the inhibitor alone would not control resistance but required an appropriate protein background within the seed). Eventually however, bruchid resistant materials for international testing were produced (Redden et al, 1984). Messina and Renwick (1985) have investigated the resistance of four selected cowpea lines derived from crosses with TVu2027 and found them to exhibit resistance through increasing the development period of C. maculatus but, unfortunately, not by causing high mortality. It is clear that introducing resistance from a donor variety may not always be straightforward, but the varieties produded by IITA represent an attempt to develop bruchid resistant varieties that was at least party successful.

At TDRI we bagan to consider the possibility of insect populations themselves developing resistance to the resistant varieties that were under development. Most of our research had been carried out using a strain of C. maculatus that had originated from Campinas in Brazil. We quickly established that a strain originating from Nigeria was already pre-adapted to be much less affected by the anti-metabolic properites of the resistant variety (Fig. 3) (Redden et al, 1983). A graduate student from Royal Holloway and Bedford New College in the U.K. was brought in to study aspects of geographical variation in strains of C. maculatus. He and his colleagues studied the Campinas, Nigerian and a Yemeni strain of the insect and found great differences in many aspects of their biology, their behaviour when infesting seeds and their ability to breed on resistant varieties of cowpeas (Dick and Credland, 1986; Credland et al, 1986). Considerable variation exists within the species C. maculatus that could result in the development

of biotypes that are able to overcome, at least partially, the resistance of newly developed cultivars of cowpea.

Beans

Beans (Phaseolus vulgaris) are the most important grain legume in South America. They are also widely grown in Africa, parts of Asia, Europe, Australia and North America. They are attacked in store by two bruchid beetles, Acanthoscelides obtectus (Say) and Zabrotes subfasciatus Boheman. TDRI and the University of Durham have recently started to collaborate with the International Centre of Tropical Agriculture (CIAT) in Colombia in the study of resistance of beans to Bruchidae. At CIAT an intensive screening of cultivated beans failed to find a single resistant line (Schoonhoven and Cardona, 1982). However, resistance was identified among samples of wild P. vulgaris from Mexico (Schoonhoven et al, 1983). At TDRI we have confirmed that one variety, G12953, is highly resistant to both A. obtectus and Z. subfasciatus (Table 2). Biochemical studies of the resistant variety at Durham have revealed several interesting chemical features of the resistant seed and bio-assays of compounds from the resistant line have demonstrated that a component of the soluble carbohydrate fraction is strongly antimetabolic to A. obtectus larvae (Fig. 4). We are at present continuing to investigate the mechanism of action of the antimetabolite, and plans are in hand to study the inheritance of resistance in crosses between the resistant wild variety and cultivated lines. It will be necessary to study the mammalian toxicity of the antimetabolite.

Improving the resistance of crops: the multi-displinary approach

The case histories outlined above serve to illustrate the multi-disciplinary aspects of studying host plant resistance. A team of scientists was involved in the work, each contributing his or her specialised talent. The original identification of resistant lines was done by scientists from crop improvement institutes utilising their knowledge of the plants and the extensive collections of breeding materials that the international centres have made available. Entomologists with specialised knowledge of storage species were involved in the testing of varieties and the development of appropriate bio-assay methods, and bio-chemists studied the natural antimetabolites of the resistant lines and purified them for testing. The eventual development of new resistant varieties depend upon the work of plant breeders who introduced desirable traits into commercial crop varieties. The storage entomologists monitored the danger of the evolution of biotypes of insects that may be relatively unaffected by the resistant varieties.

The study of resistance of crops in the laboratory can be a rewarding academic exercise. In universities and colleges it is a field that lends itself well to teaching agricultural and biology students. Over the last twenty years there has been a vast number of publications concerning post harvest insect resistance, but few have resulted in any improvement in crops available to farmers. A vast amount of detailed knowledge has been built up concerning the phenomenon of insect resistance, and much of the information obtained

has been published and is available for exploitation. Few research centres are sufficiently fortunate to have in one place all of the specialisations needed to work together to develop resistant varieties so the improvement of crop varieties is a task that lends itself to inter-institutional and international cooperation.

The potential for the improvement of resistance in crops

Cereals

A wide range of susceptibility to major storage pests has been demonstrated in all of the widely grown cereals (reviewed by Dobie, in press). Parallel selection for resistance to storage pests with selection for other criteria should result in acceptable levels of resistance. Certain characteristics desirable for storge pest resistance may be incompatible with grain characters required for other purposes: for instance, the husk (shuck) cover of maize ears is important for protection from *Sitophilus* in the field and the yield of a *Sitophilus* resistant variety may therefore potentially be reduced due to the need to ensure that the ear is not so large that it sticks out beyond the protecting leaves. In rice stored as paddy, it has been shown that grains with a very tight set of glumes covering them are resistant to attack by *Rhyzopertha dominica* while those with loose or gaping ears are susceptible (Breese, 1963). Varieties selected for easy milling may exhibit characteristics that result in high susceptibility. Especial care will have to be taken in the cases where cereals are introduced into new climatic zones or where newly developed cereal types are grown. In neither case are the crops likely to have evolved acceptable levels of resistance. For example, the new cereal triticale (derived from a wheat x rye cross) is highly susceptible to attack by all species of *Sitophilus* (Dobie and Kilminster, 1978).

Legumes

Legumes utilize secondary chemical compounds in their structures as defence mechanisms against insects, so it is probable that we will be able to exploit such compounds to develop resistant varieties. Non-protein amino acids, lectins (phytoaemagglutinins), inhibitors of digestive enzymes and tannins have all been shown to be toxic to certain insects. Extensive screening will be needed to identify varieties that are resistant and considerable effort will be needed to identify the biochemical basis of resistance. If novel antimetabolites are introduced into commercial crops, then stringent tests will be necessary to ensure that the resultant resistant lines are not harmful to consumers.

The genes responsible for the synthesis of some of the potentially useful antimetabolites could be identified relatively easily. The exciting possibility exists of then using "genetical engineering" techniques to directly transfer resistance across varietal, specific and even generic boundaries.

Acknowledgements

I am grateful to Dr A M R Gatehouse for her collaboration in all aspects of the biochemistry of insect resistance.

Table 1. Response of several cultivars of cowpea to *Callosobruchus maculatus*

Variety	Total number eggs laid	Total number adults emerged	% adult emergence ± 1 s.d.
TVU37	116a, b	110	86.6 ± 4.3a
TVu57	105b,c	96	91.7 ± 6.7a
TVu76	116a,b	104	90.0 ± 2.5a
TVu1190E	168a	150	89.0 ± 6.0a
TVu1502-ID	1512a	124	92.0 ± 6.0a
TVu2027	105b,c	0	0.0 ± 0.0b
TVu 3629	80c	62	90.6 ± 9.5a
TVu4457	85c	81	95.1 ± 6.2a
Farrin Wake	83c	74	89.0 ± 4.9a

All figures followed by the same letter are not significantly different at the 5% level of significance (Duncan's Multiple Range Test).

Table 2. Response of several wild bean varieties to *Acanthoscelides obtectus* and *Zabrotes subfasciatus*.

	Acanthoscelides obtectus		*Zabrotes subfasciatus*		
Variety	% development to adult	Mean development period	% development to adult	Mean development period	Mean eggs per seed
G12953	1.5	56.6	19.6	53.2	16.3
G12942	35.0	35.0	78.3	49.2	29.7
G10011	50.4	31.0	81.7	35.4	21.7
G12896	52.0	36.6	71.4	46.4	22.9
G10019	59.0	31.3	74.5	34.7	19.6
G10030	67.5	31.4	84.2	34.2	26.5
G13014	70.7	28.3	81.1	33.6	28.4

Figure 1. Grouped indices of susceptibility for maize varieties collected from Sahelian Africa and from The Caribbean and Central Mexico.

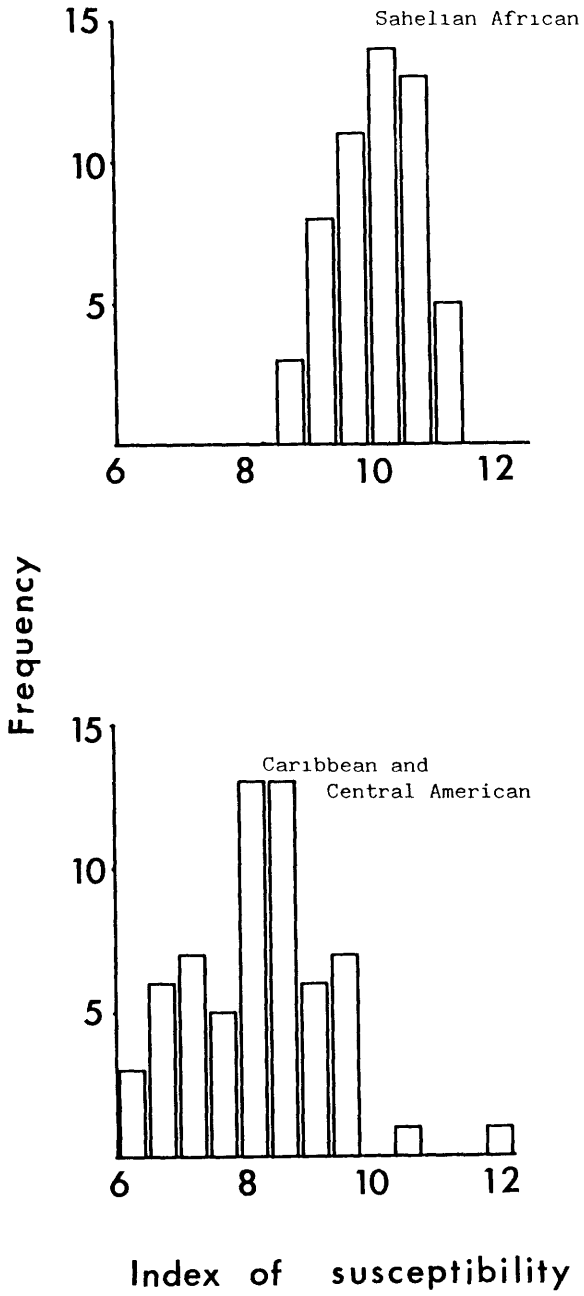


Figure 2. Survival of Callosobruchus maculatus on diets incorporating extracts of cowpea variety TVu2027.
a: Control (meal of susceptible cowpea variety TVu57).
b: Meal of resistance cowpea TVu2027. c: Control + 10% albumin proteins. d. Control + 10% globulin proteins.
e: Control + 10% albumin proteins minus trypsin inhibitor.
f: Control + 0.1% trypsin inhibitor. g: Control + 0.5% trypsin inhibitor. h: Control + 0.8% trypsin inhibitor.
i: Meal of susceptible cowpea variety TVu57.
Treatments c - i were based on cowpea meal that had been autoclaved to remove endogenous trypsin inhibitory activity.
(Adapted from Gatehouse et al, 1979).

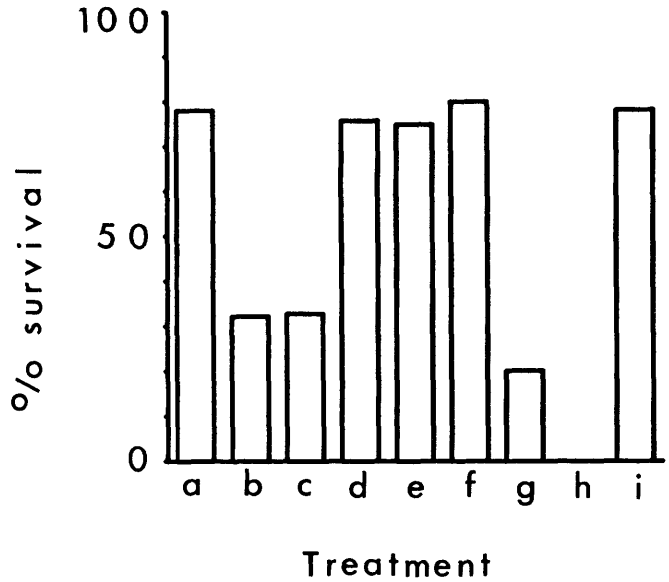


Figure 3. Development of Campinas (Brazil) and IITA (Nigeria) strains of Callosobruchus maculatus on resistant cowpea variety TVu2027 and on susceptible "black-eyed peas" (BEP). From Redden et al (1983).

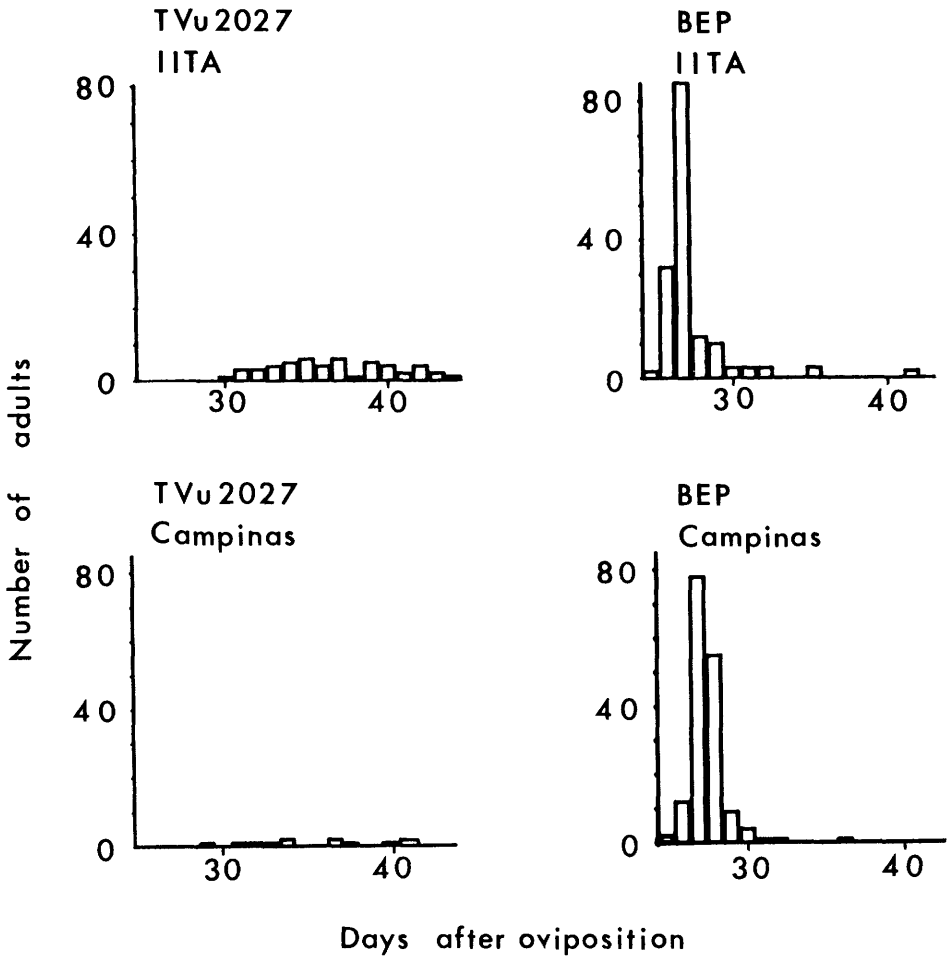
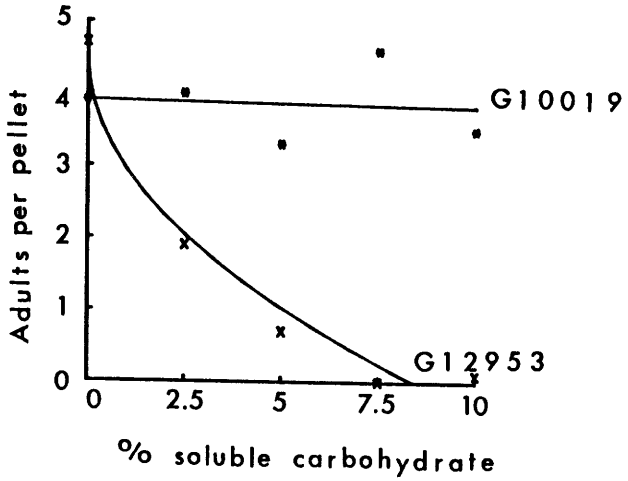


Figure 4. The response of Acanthoscelides obtectus to artificial diets incorporating a soluble carbohydrate fraction extracted from resistant wild bean variety G12953.

A



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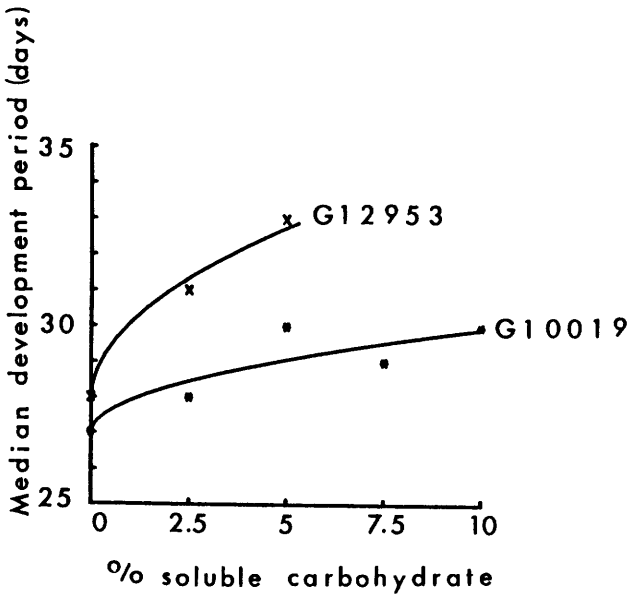

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