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VARIETAL RESISTANCE

*James E. Throne, James E. Baker, Frank J. Messina,
Karl J. Kramer, and John A. Howard*

1.0 BACKGROUND AND HISTORY

Production of all classes of wheat grown in the United States totaled 2.5 billion bushels in 1997. Common wheat, *Triticum aestivum* L. subsp. *aestivum*, is the primary source of all wheat varieties grown in the United States, with over 200 varieties known and over 100 varieties cultivated. These 100 varieties constitute over 95% of all wheat cultivated in the United States. Five general wheat classes are predominant in the United States: hard red winter, hard red spring, soft red winter, white, and durum.

Corn, *Zea mays* L., is indigenous to the Americas and is the dominant cereal grain in the United States with 9.4 billion bushels produced in 1997. Rice, *Oryza sativa* L., is the major food cereal for much of the world, particularly the Asian continent. The United States produces only about 1% of the total rice crop (179 million bushels in 1997), but it is a leading exporter with an export value of nearly \$1 billion in 1997. Production of barley, *Hordeum vulgare* L., in the United States in 1997 was 375 million bushels. Sorghum, *Sorghum bicolor* (L.) Moench, is the world's 5th leading cereal in terms of acreage planted and total production, behind corn, wheat, barley, and rice. Sorghum is highly adapted to heat and water stress and can be grown in dry regions where other cereals cannot compete. About 80% of the total acreage planted to sorghum is in Africa and Asia. About 9.4 million acres are planted to sorghum in the United States. Average yields of sorghum in the United States, however, are about 4.5 times greater than yields in Africa. Production in the United States in 1997 was 653 million bushels. The United States dominates world exports of sorghum, accounting for 70-80% of the trade. The production of oats, *Avena sativa* L., in the United States in 1997 was 176 million bushels. Triticale is a cross between wheat (*Triticum*) and rye (*Secale*) that combines the high protein content of wheat with the high lysine content of rye.

Grain legumes provide an important source of protein for much of the world's population and serve to supplement low-protein cereals and root crops (Singh and Singh 1990). They can be roughly divided into pulses, which are usually consumed directly, and oilseeds, which are either eaten directly or exploited for their high content of extractable lipids (Smartt 1990). Most taxonomists divide legumes (Family Fabaceae) into 3 subfamilies: the Caesalpinioideae, Mimosoideae, and Papilionoideae (Figure 1). Grain legumes all belong to the Papilionoideae, as do the major forage legumes (Smartt 1990). Within the Papilionoideae, most pulses are found in the tribe Phaseoleae and are therefore closely related, but economically

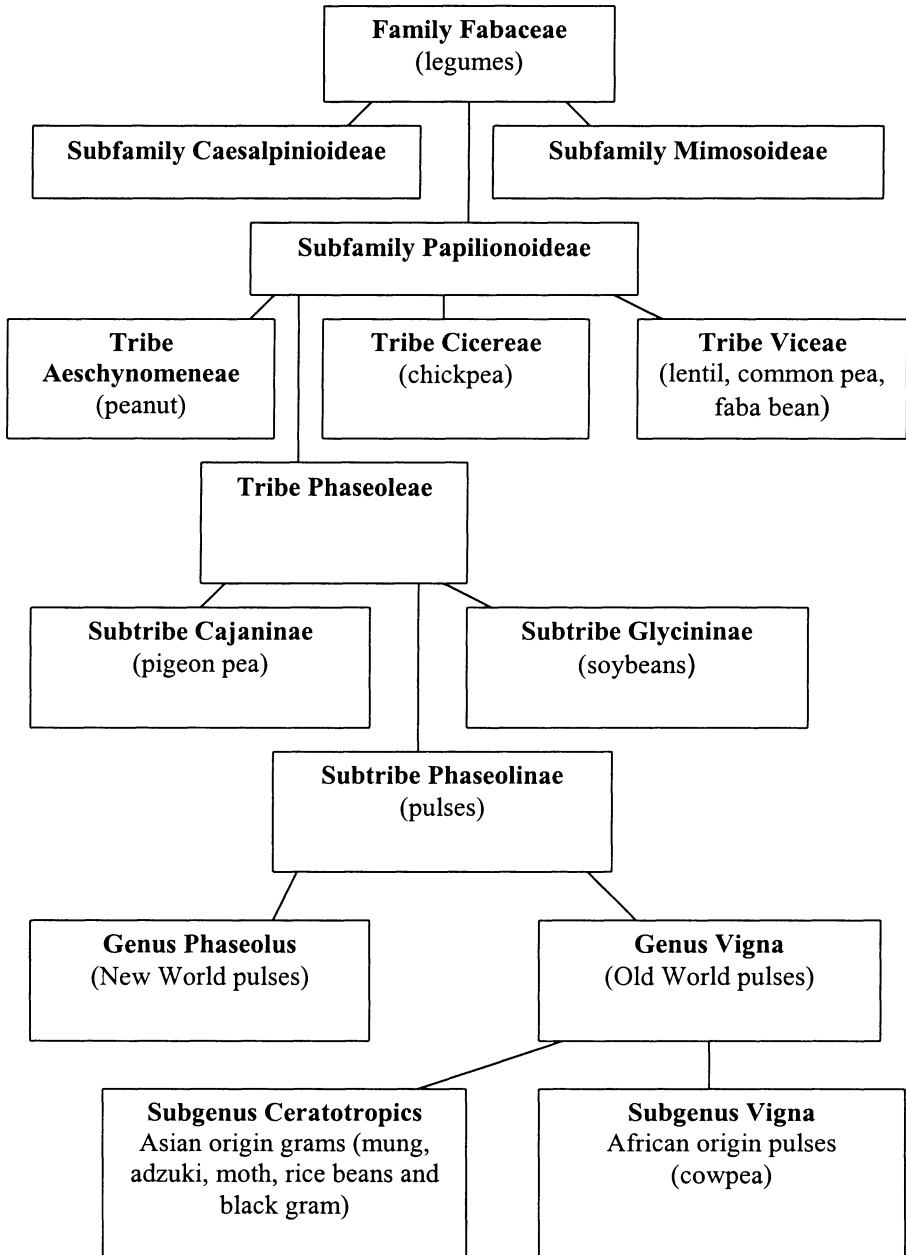


Figure 1 Taxonomic classification of legumes

important species also occur in the tribes Aeschynomeneae (peanut), Cicereae (chickpea), and Viceae (lentil, common pea, faba bean). The tribe Phaseoleae includes New-World pulses (beans in the genus *Phaseolus*) and Old-World pulses (*Vigna* species) in the subtribe Phaseolinae, soybean (subtribe Glycininae), and pigeon pea (subtribe Cajaninae). Within the genus *Vigna*, we can also distinguish African-origin pulses (subgenus *Vigna*, including cowpea) from "grams" indigenous to Asia (subgenus *Ceratotropis*), such as mung bean, adzuki bean, black gram, moth bean, and rice bean (Smartt 1985, Tateishi and Ohashi 1990).

1.1 Major insect pests

Most cereals are susceptible to serious insect attack during storage and throughout marketing channels. A complex of primary and secondary insect pests can cause loss of wheat grain quality during storage. The major primary pests, and the insects against which most wheat varietal resistance tests are conducted, include the lesser grain borer, *Rhyzopertha dominica* (F.); Angoumois grain moth, *Sitotroga cerealella* (Olivier); rice weevil, *Sitophilus oryzae* (L.); maize weevil, *Sitophilus zeamais* Motschulsky; and granary weevil, *Sitophilus granarius* (L.). Adults of *R. dominica* and *S. cerealella* lay eggs singly or in batches outside of the grain. After eggs hatch, larvae penetrate and feed internally within the grain. In contrast, adult female *Sitophilus* species oviposit into small holes that they bore into the kernel. After eggs hatch, the weevil larvae also feed and develop within the grain. Secondary pests of wheat, pests which generally cannot damage intact kernels, include the Indianmeal moth, *Plodia interpunctella* Hübner; flat grain beetle, *Cryptolestes pusillus* (Schönherr); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); confused flour beetle, *Tribolium confusum* Jacquelin du Val; and red flour beetle, *Tribolium castaneum* Herbst. These secondary pests generally lay eggs outside of grain kernels or in cracks in kernels. The larvae feed on broken kernels, although some can feed on the germ of intact kernels.

The insects most commonly found in stored corn are the primary pests, *S. zeamais* and *S. cerealella*, and the secondary pests, *C. pusillus*, *C. ferrugineus*, *P. interpunctella*, the almond moth [*Cadra cautella* (Walker)], *O. surinamensis*, the foreign grain beetle [*Ahasverus advena* (Waltl)], *T. confusum*, *T. castaneum*, and the hairy fungus beetle [*Typhaea stercorea* (L.)] (Arbogast and Throne 1997, Sedlacek et al. 1998). Primary pests of sorghum are *S. zeamais*, *S. oryzae*, and *S. cerealella*. Primary pests of rice are *R. dominica*, *S. cerealella*, *S. oryzae*, and *S. zeamais*. Generally, *R. dominica* and *S. cerealella* are much more damaging to rice than *Sitophilus* species, with *R. dominica* causing the most weight loss but damage from *S. cerealella* resulting in highest loss of milling yield (Cogburn 1977a).

The insects infesting barley are similar to those infesting wheat (Gardner et al. 1988, Buchelos and Katopodis 1995). Secondary pests are the most common insects found in oats stored in the United States, particularly *O. surinamensis*, *Cryptolestes* and *Tribolium* species. (Storey et al. 1983, Ingemansen et al. 1986). However, primary insect pests can also infest oats (Horton 1982). Triticale is similar to wheat in supporting insect growth (White and Loschiavo 1988) and, thus,

is susceptible to many of the same insect pests as wheat (Dolinski et al. 1971, Greening 1983).

There are believed to be at least 2,000 species of seed beetles associated with leguminous and non-leguminous hosts (Southgate 1979). In the typical life cycle, eggs are laid on the walls of developing legume pods or on seeds within dehisced pods. After hatching, the larva burrows into a seed and completes its development. Females in most species accept only one or a few legume species for oviposition (Janzen 1980), and an obligate diapause stage limits non-pest and field-pest species to 1-2 generations per year. In contrast, a small number of bruchids (<20 species) have become occasional or severe storage pests because they exhibit a broad host range, readily lay eggs on threshed, mature seeds, and pass through several generations without an intervening diapause. Pre-harvest infestation is typically low (Larson and Fisher 1938, Hagstrum 1985, Germain et al. 1987), but exponential population growth in storage causes consumption of nearly all of the cotyledon mass, making seeds unfit for either consumption or germination. Infested seeds are easily recognized by multiple exit holes left by emerging adult beetles.

Some bruchid pests display a polymorphism that further enhances their ability to exploit grain legumes (Utida 1972). In the cowpea seed beetle, *Callosobruchus maculatus* (F.), adults emerge either as a fecund, sedentary morph, which is well suited to attack seeds after harvest, or as an "active" morph, which delays reproduction, disperses from stores, and lays eggs on pods in the field (Messina and Renwick 1985a). This polymorphism permits efficient cycling between storage and field environments, and may have evolved within the several thousand years that humans have stored legume seeds. An individual's path of development depends on its genotype and the level of crowding it experienced as a larva (Sano-Fujii 1986, Messina 1987).

The most important storage bruchids belong to two Old-World genera, *Callosobruchus* and *Caryedon*, and two New-World genera, *Acanthoscelides* and *Zabrotes*. Four species chronically cause substantial losses (Birch et al. 1985): *C. maculatus*; the adzuki seed beetle, *Callosobruchus chinensis* (L.); the bean seed beetle, *Acanthoscelides obtectus* (Say); and the Mexican bean seed beetle, *Zabrotes subfasciatus* (Boheman) (each species is also referred to as a "weevil", but this convention is discouraged to prevent confusion with true weevils [Curculionidae]). International movement of infested seeds has greatly expanded the geographic range of each species. Although Old-World species tend to attack *Vigna* species and New-World species are associated with *Phaseolus* species, both *C. maculatus* and *Z. subfasciatus* can now be regarded as cosmopolitan pests of legumes indigenous to each region (Birch et al. 1985, Meik and Dobie 1986). Some bruchids, such as *Bruchidius atrolineatus* (Pic), cannot be simply classified as either a storage or field pest because they show some population growth in storage before undergoing diapause (Germain et al. 1987).

1.2 Bioassay methods and evaluation of resistance

Varieties that express resistance have physical or biochemical attributes that modify behavioral responses (xenobiosis) or that adversely affect development or

survival of the pest insect species through metabolic aberrations (antibiosis). To determine mechanisms, bioassay parameters can be associated with measured physical or biochemical properties through statistical correlation. Most evaluations of varietal resistance in cereals have been conducted with no-choice bioassays in which adult insects are confined with samples of each tested variety. Recently, an artificial seed bioassay method has been developed in which biochemicals of interest can be incorporated into an artificial seed and tested for its effect on insect development (Pittendrigh et al. 1997). Whether choice tests or no-choice tests are conducted, standardization of bioassay methods for each pest insect is essential during evaluation of varieties (Bhatia 1978). It is particularly important that moisture contents of the varieties be controlled. Also, insect density can influence ovipositional responses among different variety samples. For example, optimum ovipositional response of *S. oryzae* in wheat is about 6-8 eggs per female per day (Longstaff 1981). As insect-insect interactions increase, ovipositional response becomes much lower, and this could interfere with varietal comparisons. Baker et al. (1991a) evaluated 30 Eastern soft wheat cultivars by allowing 5 female *S. oryzae* to oviposit for 3 days on 25-gram samples of each cultivar. Under these conditions, progeny production was 7.2 weevils per female per day, a near optimum response.

The most common parameter used to compare varieties is total progeny production (Horber 1983). Other methods include the Dobie Index, which takes into account both total progeny production and development time (Dobie 1974). These methods in themselves, however, do not provide information on how differences in varietal susceptibility or resistance may translate into differences in population development of the pest insects during storage. Test parameters can be included in population models that can be used to predict dynamics of insect population growth under various storage conditions (Throne 1989, Baker et al. 1991b).

The International Rice Research Institute (IRRI, Laguna, Philippines) has developed standardized protocols to evaluate rice varieties against *S. oryzae*, *R. dominica*, and *S. cerealella* (Heinrichs et al. 1985). Two series of tests are conducted: an initial screening to detect varieties that are relatively resistant and a second series of tests to determine the mechanism of resistance. For initial screening against *Sitophilus* species and *R. dominica*, 30-gram samples are infested with 15 pairs of weevils or borers for a 7-day oviposition period. Total progeny production and sample weight loss are determined and compared with varieties known to be susceptible. For initial screening against *S. cerealella*, 200 eggs oviposited onto paper are placed on 10-gram samples of rice. After 7 or 14 days, egg hatch is determined. The number of emerging adults and damaged grains, as well as total weight loss of grains, is determined.

Varieties that show moderate or high resistance are evaluated further with bioassays designed to determine whether antixenosis or antibiosis modulates resistance. For *Sitophilus* species, choice tests and ovipositional responses are used for evaluating antixenosis. Weevil weight, progeny production, and an index of susceptibility are used as antibiosis bioassays. Antixenosis for oviposition by *S. cerealella* is determined during choice bioassays. Assays for antixenosis for feeding or mechanical prevention of entry into the rice grain and for antibiosis are

set up as described by Russell and Cogburn (1977) and consist of determining percentage egg hatch, survival to adult, and mean time to emergence of adults.

Methods to screen plant genotypes for resistance to bruchids are generally straightforward, in part, because the laboratory presents a good approximation of the storage environment and because the insects have short generation times (approximately one month at 25°C). However, a careful selection of protocols is often needed to determine the precise mode of resistance (Dobie et al. 1990, Ofuya and Credland 1995a). Most bioassays use mature, dried seeds, but even infestations by "storage" bruchids begin with egg-infested pods in the field. Characteristics of the pod, thus, should not be overlooked as potential sources of resistance. Moreover, cultivars with highly susceptible seeds may possess pod traits that reduce pre-harvest infestation (Talekar and Lin 1981, Fitzner et al. 1985).

Non-preference resistance can be assessed by recording egg densities on seeds or pods after a standard period of exposure to egg-laying females. Although potential hosts may be presented in either choice or no-choice arenas, the latter protocol will provide a more realistic estimate of resistance in storage. As described below, both chemical and physical properties of seeds have been shown to influence host acceptance by egg-laying females. Distinguishing between physical and chemical factors can be accomplished by applying chemical extracts to uniform, artificial hosts, such as glass beads. Credland and Wright (1988) showed that *C. maculatus* beetles laid as many eggs on beads dipped in an extract of a susceptible cowpea as they did on the cowpeas themselves. This technique can help identify chemicals that either stimulate or deter egg laying; these chemicals may then be used to manipulate female behavior.

Because pulse beetles deprived of preferred hosts eventually lay eggs on less acceptable hosts (or on other substrates) (Messina et al. 1992), factors conferring antibiosis resistance are generally more promising than those that merely deter oviposition. Antibiosis is usually manifested in a reduction in larval survival, a delay in development, or a decrease in the weight of emerging adults. A simple way to detect antibiosis would be to infest seed batches with a standard number of egg-laying females, note the number of eggs per seed, and compare this with the number of exit holes produced by the progeny (Ahmed et al. 1989). Additional information can be gained by estimating the decrease in seed weight after progeny have emerged. Such trials, however, do not allow one to separate the contributions of delayed larval development versus increased larval mortality in reducing pest population growth. Because some resistant cultivars slow development with little effect on mortality, bioassays should be carried out for much longer than the length of a generation on a susceptible host (Ofuya and Credland 1995a). Estimates of adult mass at emergence are also useful in bioassays because mass is correlated with the amount of seed consumed per larva and with the potential fecundity and longevity of emerging females (Credland and Dick 1987, Credland and Dendy 1992).

Seed resistance to bruchids may depend on properties of the seed coat (testa), the underlying cotyledons, or both (Janzen 1977). Beetles chew through but do not typically consume testae (Southgate 1984), which could present a mechanical barrier or contain deterrent or toxic chemicals. Separating resistance in the testa

from that in the cotyledons can be accomplished by microscopic observations of whether death occurs before or after larvae reach cotyledon tissue (Messina 1984), but such observations are not practical for large-scale screenings. A better approach is the use of artificial seeds made from cotyledon tissue alone (Osborn et al. 1988, Cardona et al. 1989). Shade and colleagues (Shade et al. 1986, Murdock et al. 1988) have been particularly successful in isolating resistance factors using this technique. Their artificial seeds were derived from milled flour that is made into a paste. The paste can be supplemented with chemicals suspected of conferring resistance, injected into molds, and lyophilized. Beetle performance on such seeds is only slightly lower than that on intact seeds. This work has also revealed the importance of using purified chemicals in bioassays of legume resistance (Huesing et al. 1991).

1.3 Physical and biochemical mechanisms of resistance

Extensive varietal testing for insect resistance in wheat has been conducted in India, where between 1965 and 1970, a total of 1,948 varieties were tested (see Bhatia 1976). Among these varieties, 53 were relatively resistant to *S. oryzae* and 48 were relatively resistant to *R. dominica*. Ram and Singh (1996) evaluated 64 wheat varieties with no-choice bioassays and found differing degrees of susceptibility to attack by *S. oryzae* and *R. dominica*. Several of the Indian wheats are relatively resistant and show promise for future development. El-Nahal et al. (1982) found that 2 of 4 Egyptian wheat varieties tested against *S. oryzae* and *S. granarius* were relatively resistant, whereas Irshad (1988) found no significant resistance against *S. oryzae* among 6 varieties grown in Pakistan. Boles and Ernst (1976) found 2 varieties, among 6 United States varieties tested, that were less susceptible to *S. oryzae*, while McGaughey et al. (1990) found significant differences in susceptibility among wheat classes grown in the United States. An experimental high-yielding hard wheat variety was the most resistant of 23 Australian wheats tested against *S. oryzae* and *R. dominica* (Amos et al. 1986). Most common Canadian wheat varieties were susceptible to stored-grain insect pests, but hard wheats were less susceptible than soft wheats (Sinha et al. 1988).

Evers and Bechtel (1988) present an excellent review of morphological features of developing and mature wheat grain. Physical features of the pericarp and seed, along with biochemical components of these structures that elicit behavioral responses of stored-product insects (Baker and Loschiavo 1987), affect the degree to which insects can utilize this cereal fruit for growth and development.

Compared with other cereals, mechanisms of varietal resistance in wheat are poorly understood (Horber 1983). Grain size, hardness, protein content, and oil content have been most commonly analyzed when evaluating resistance mechanisms in wheat. McGaughey et al. (1990) evaluated grain from 62 United States wheat varieties for insect susceptibility. Although all of the wheats tested were susceptible to insect damage, there were significant differences among wheat classes in their degree of susceptibility. However, there were no significant differences in susceptibility among varieties within a given class. Singh et al. (1974) and Ram and Singh (1996) found that *S. oryzae* preferred to oviposit on

varieties with larger grains, whereas McGaughey et al. (1990) found that grain size was not useful for evaluating resistance against either *S. oryzae* or *R. dominica*. Grain texture has also been implicated, with smoother grain surfaces preferred (Tyagi and Girish 1975). *Sitophilus* species prefer to oviposit in the brush end of wheat grain (Bishara 1967), even though the seed coat in that region is about 10-fold harder than that in germ (Sinha and Voisey 1978). Germ is toxic to first stage weevil larvae (Gomez et al. 1982), so chemical factors may also be involved in avoidance of the germ end during oviposition in these species.

Effects of wheat grain hardness, commonly associated with resistance to stored-product insect damage, are not clear-cut. McGaughey et al. (1990) found that large differences in hardness, such as those between wheat classes, correlated with the number of *S. oryzae* progeny, but that smaller differences were not correlated with resistance and did not affect production of *S. oryzae*. These results were similar to those of Khokhar and Gupta (1974), Amos et al. (1986), and Sinha et al. (1988). Ram and Singh (1996), however, found that grain hardness exhibited the closest relationship with susceptibility to *S. oryzae* among the 64 varieties tested.

Varietal protein content was negatively associated with progeny production of *S. oryzae* (Amos et al. 1986, Ram and Singh 1996), whereas Khokhar and Gupta (1974) found no significant correlation between protein content and total weevil progeny. Ram and Singh (1996) also found that oil content was not a factor, whereas crude fiber was negatively correlated with susceptibility. Baker et al. (1991a) found a positive correlation between α -amylase inhibitor content and development of *S. oryzae* on 30 Eastern soft wheat varieties. The differences in development time, however, were small.

It is apparent that resistance mechanisms among wheat varieties have not been elucidated and that additional studies are needed. Ram and Singh (1995) found evidence that resistance to *S. oryzae* in one variety was polygenic, indicating that multiple factors are responsible. Elucidation of these factors will be difficult because resistance is a relative term and is generally not clear-cut. As an example of the difficulty involved, over 100 volatile compounds have been analyzed in wheat samples from Kansas (Seitz 1995). However, no differences in composition of these volatiles were found among the 5 cultivars tested. If olfactory cues are involved in varietal susceptibility, there was no genetic variation in these cultivars that could be exploited or used to modify behavioral responses involved in host utilization.

Resistance to *S. zeamais* and the larger grain borers, *Prostephanus truncatus* (Horn), in corn has been correlated with ferulic acid content of the corn (Classen et al. 1990, Arnason et al. 1997). Esterified ferulic acid forms cross-links to cell wall arabinoxylans, and may be partly responsible for kernel hardness, which also is correlated with insect resistance (Arnason et al. 1997). Unbound ferulic acid in the aleurone layer may be astringent and may act as an antifeedant to insects (Arnason et al. 1997).

Damage to stored corn by postharvest insect pests may increase infection by the aflatoxin-producing fungus, *Aspergillus flavus* Link ex Fries, by providing a path for entry into the kernel and by increasing moisture content of the corn (Sinha and Sinha 1992). Therefore, development of corn lines that are resistant to stored-

product insect pests should also reduce aflatoxin contamination of stored corn. Throne et al. (1995) identified corn lines that were relatively resistant to both maize weevils and *A. flavus*. Of the chemical factors measured, only lipid content was correlated (negatively) with resistance to maize weevils. There was no relationship between resistance to maize weevils and phenolic acid content in these corn lines.

Rice is harvested as a covered grain that is enclosed in a husk or hull (Juliano and Bechtel 1985). The hulled grain is termed rough rice or paddy. Dehulled rice is termed brown rice. The hull has a high silica content and constitutes about 20% of grain weight. Two modified leaves, the palea and lemma, form the hull, which surround the caryopsis. The tightness of the hull is dependent upon the degree to which the palea and lemma are held together by two hook-like structures. Tightness of the hull is a major resistance mechanism against stored grain insect attack on rough rice, and harvesting methods that minimize damage to rough rice can eliminate or reduce subsequent insect damage during storage (Juliano 1981).

Bhatia (1976), Russell and Cogburn (1977), Tyagi and Girish (1977), Juliano (1981), and Horber (1983) have reviewed many of the varietal testing studies and have discussed the main mechanisms of resistance in rice to stored-grain insects. The first major study of resistance in rice was conducted by Breese (1960). He demonstrated that *S. oryzae* could not attack rice grains with intact hulls. He also found that larvae of *R. dominica* could attack rough rice if there are any cracks, even minute cracks, between the palea and lemma. Breese concluded that the degree of insect infestation in stored paddy is dependent upon the number of grains with broken or damaged hulls.

Subsequent to Breese's (1960) study, the importance of varietal resistance as a component of integrated management of insect pests became more important, and the number of studies evaluating rice varieties and accessions increased. Russell (1968) and Cohen and Russell (1970) found that the infestation of rice grains by *Sitophilus* species and *S. cerealella* was related to number of gaping hulls or broken palea and lemma. Cogburn (1974) confirmed the importance of hull characteristics in resistance and found that weevils and borers developed equally well on brown rice prepared from rough rice varieties that were resistant. Evidence from Cogburn's (1974) study also indicated that additional unknown factors were involved in the resistance. Cogburn (1977b) evaluated 111 rice varieties and found 32 varieties expressing a significant level of resistance to *S. cerealella*. Russell and Cogburn (1977) expanded these latter studies and evaluated 780 varieties of rice from the United States Department of Agriculture World Collection and also found evidence for 2 resistance bases operating against *S. cerealella*. Both resistance bases involved prevention of newly hatched larvae from entering the kernel. Cogburn et al. (1983) found that larvae of *S. cerealella* could infest rough rice by penetrating through the central vascular bundle in the abscission scar. As a result of these studies, it was concluded that there are at least 2 hull characters that impart insect resistance during storage, and both can be used in rice breeding programs.

In addition to hull characteristics in rough rice, studies have been conducted to evaluate resistance characters in brown rice. Rout et al. (1976) found that grain hardness was negatively correlated with susceptibility of 8 varieties to *S. oryzae*. Protein and starch contents of the 8 varieties were not significantly correlated with

resistance. Morallo-Rejesus et al. (1982) selected 15 rice lines from the IRRI breeding program with a broad range of protein and amylose contents and evaluated these lines for resistance against *S. zeamais*, *R. dominica*, and *T. castaneum*. In addition to protein and amylose, varieties were also analyzed for grain size, grain hardness, gelatinization temperature (as measured by alkali spreading value), and gel consistency. In these tests, the indices of susceptibility of the varieties to the 3 insect species were not significantly related. For *S. zeamais*, grain size was positively correlated and grain hardness was negatively correlated to the susceptibility index. Protein content, amylose content, and alkali spreading value were not significant. In an evaluation of 22 Chinese varieties/lines, Zhang and Deng (1993) found that *S. zeamais* laid more eggs on varieties with larger and smoother grains. High amylose content favored development, whereas high protein content was detrimental to *S. zeamais* growth.

Sorghum, commonly called milo in the central United States, is a member of the grass family, Poaceae. Serna-Saldivar and Rooney (1995) describe the structure of the sorghum kernel in detail. The sorghum grain, considered to be a naked caryopsis, is composed of an outer layer or pericarp, seed coat or testa, endosperm, and germ. Depending on variety, the testa can be pigmented and can contain differing amounts of condensed tannins. Varieties with high tannin content (type III sorghums) are grown where bird predation is a serious problem. Tannins contribute to the astringency of sorghum and can adversely affect the bioavailability of nutrients within the grain (Klopfenstein and Hosney 1995).

Threshing of sorghum removes the glumes (hull) that surround the caryopsis. Varieties with intact glumes are nearly immune to attack by *S. zeamais* (Rogers and Mills 1974). However, unthreshed sorghum was more susceptible to attack by *S. cerealella*, whereas threshing that produced complete or partial removal of the glumes led to an increase in susceptibility to *S. oryzae* (Wongo and Pedersen 1990). Primarily farmers at the subsistence level store sorghum with glumes intact (in head), whereas nearly all sorghum in the United States is threshed.

Adetunji (1988) studied 21 Nigerian varieties of sorghum and found evidence for two modes of resistance against *S. oryzae*, reduced oviposition (non-preference) and increased mortality of larvae within the seeds (antibiosis). In addition, larval development was significantly slower in the resistant varieties. Factors responsible for these effects were not identified. Where evaluated, sorghum varieties with increasing grain hardness or increased thickness of the corneous layer of the endosperm are much less susceptible to the primary grain pests *S. oryzae*, *S. zeamais*, and *Sitotroga cerealella* (Russell 1962, 1966; Doraiswamy et al. 1976, Fadelmula and Horber 1983; Wongo 1990). Although there is some evidence that varieties with high tannin content cause reduced oviposition by *S. oryzae* (Russell 1962), Fadelmula and Horber (1983) found that tannin content was not significantly related to progeny production by *S. oryzae* on 12 tested varieties. Seed size also affects susceptibility (Russell 1962), and larger seeds produce larger insects (Wongo 1990).

In addition to antinutritive compounds such as tannins, sorghum contains a complex of proteinase inhibitors including trypsin, chymotrypsin, and elastase inhibitors (Klopfenstein and Hosney 1995). To our knowledge, proteinase

inhibitors have not been examined as a possible source of varietal resistance in sorghum to stored-product insects.

Barley may be more susceptible to stored-product insects than corn, rice, or wheat (Baker 1988), although this trend is not consistent due to varietal differences within a cereal crop (Sinha 1971, Dobie and Kilminster 1978). Hulless cultivars are more susceptible to infestation than those with hulls (Sinha 1971, Boles and Pomeranz 1979). Tightness of the hull may decrease susceptibility, although chemical factors in the hull (perhaps silica) may decrease oviposition or affect larval development (Boles and Pomeranz 1979). Oviposition is reduced when the hull is intact (Teotia and Singh 1968).

Insect population levels are reduced at lower oat moisture contents (Ingemansen et al. 1986). Older oat cultivars were covered with a protective hull that provided protection against insect pests. Newer, hulless cultivars, which lose their hull during threshing, are susceptible to infestation by the primary pests *Sitophilus* species and *R. dominica*, with twice as many *Sitophilus* species produced on hulless oats (Sinha 1969, 1971). Oats are generally more resistant to primary pests than wheat (Sinha 1969, 1971).

Triticale is similar to wheat in supporting insect growth (White and Loschiavo 1988) and, thus, is susceptible to many of the same insect pests as wheat (Dolinski et al. 1971, Greening 1983). Triticale is more susceptible to *Sitophilus* species than corn, barley, or wheat (Dobie and Kilminster 1978). Triticale varieties grown in or developed for climates similar to the southeastern United States vary little in their high susceptibility to *S. oryzae* and *R. dominica* (Baker et al. 1991b). As with other stored grains, lowering the moisture content of the grain (Baker et al. 1991b) can significantly reduce insect development on *Triticale*. *Triticale* may be highly susceptible to primary storage pests because of the soft, floury endosperm (Dobie and Kilminster 1978).

Most commercial pulses are quite susceptible to at least one bruchid species in storage, and large-scale screenings have typically identified only a few cultivars with even mild resistance (Schoonhoven et al. 1983, Dongre et al. 1996). Wild or semi-cultivated relatives of these crops ("land-races") appear to possess a wider range of resistance traits (Birch et al. 1985, Ofuya 1987). Several authors have argued that these crop relatives have been under-exploited in programs to incorporate resistance by either classical or transgenic methods (Birch et al. 1985, Marconi et al. 1997).

Physical sources of resistance may be less promising than chemical sources, but physical properties of pods and seeds can provide resistance via both non-preference and antibiosis. Females of *C. maculatus* have long been known to prefer smooth, intact seeds over those with rough, broken, or poorly filled testae (Booker 1967, Nwanze and Horber 1976). The smoothness and pubescence of the pod wall can similarly deter oviposition (Fitzner et al. 1985). Increased larval mortality in bruchids has been associated with thick-walled pods as well as those in which there is a cavity between the pod wall and the seed. This gap induces mortality because the legless, neonate larva cannot traverse it to get to the underlying seed (Ouedraogo and Huignard 1981, Messina 1984). Because cowpeas are often stored as dried pods, resistance to breakage is also a desirable pod trait (Kitch et al. 1991). The

thickness and hardness of the testa may provide some resistance (Talekar and Lin 1981), but the resistance will be most effective if combined with a thick pod wall. Together, the 2 traits may cause exhaustion or starvation of larvae before they are able to reach the cotyledon (Kitch et al. 1991).

Although a wide range of biochemical sources of resistance to bruchids has been identified, among them alkaloids, saponins, and non-protein amino acids (Gatehouse et al. 1990), most recent work has focused on a series of seed proteins that can greatly reduce pest growth. These proteins are structurally and evolutionarily related, and include lectins (phytohemagglutinins), arcelins, α -amylase inhibitors, and protease inhibitors. There has been considerable controversy over which compounds confer resistance in specific plant-insect combinations. For example, a screening of thousands of cowpea varieties in the 1970s yielded only a single landrace (designated TVu 2027) with significant resistance to *C. maculatus* (Singh and Singh 1990). Resistance was originally ascribed to a trypsin inhibitor (Gatehouse and Boulter 1983), which could account for the delayed development of larvae in seeds of TVu 2027 and its derivatives (Messina and Renwick 1985b, Ofuya and Credland 1995a). Although cowpea trypsin inhibitor is toxic to several insects (Hilder et al. 1987), subsequent studies have cast doubt on its importance in resistance to bruchids (Baker et al. 1989, Xavier-Filho et al. 1989, Zhu et al. 1994).

Early claims that phytohemagglutinins confer resistance to *Callosobruchus* in *Phaseolus* beans (Janzen et al. 1976, Gatehouse et al. 1984) have also been challenged (Huesing et al. 1991). Alpha-amylase inhibitors are more likely responsible for the poor performance of this genus on New-World pulses (Ishimoto and Kitamura 1989). By inhibiting α -amylase activity in the midgut, these compounds significantly delay larval growth. Nevertheless, α -amylase inhibitors probably do not account for all resistance to *Callosobruchus* in cowpeas (Reis et al. 1997). Recent evidence suggests that storage proteins called vicilins may confer resistance in cowpea by virtue of their low digestibility and their binding with chitin in the beetle midgut (Yunes et al. 1998). Yet another class of compounds, cyclopeptide alkaloids called vignatic acids, may mediate resistance to *Callosobruchus* in Asian pulses such as mung bean (Kaga and Ishimoto 1998).

Because phytohemagglutinin is found in most beans (*Phaseolus* species), natural concentrations are clearly ineffective against New-World seed beetles such as *Acanthoscelides* and *Zabrotes*. Instead, arcelin proteins in wild *Phaseolus* species have been implicated as sources of resistance to both *Zabrotes* and *Acanthoscelides* (Osborn et al. 1988, Hartweck et al. 1997). The insecticidal mechanism is unknown in this case, but the ratio of arcelin to another seed protein (phaseolin) appears to determine resistance levels (Minney et al. 1990). Osborn et al. (1988) suggested that arcelin is toxic to *Z. subfasciatus*, but Minney et al. (1990) emphasized beetle starvation because of reduced digestibility. Separating toxic effects from effects of reduced nutritional quality is often difficult in assays of legume seeds (Yunes et al. 1998). Although the specific α -amylase inhibitor (α AI-1) that reduces the performance of *Callosobruchus* in beans is ineffective against New-World bruchids, a variant (α AI-2) has recently been isolated that inhibits amylases in the midgut of *Zabrotes* (Grossi de Sa et al. 1997, Chrispeels et al.

1998). Combining this compound with arcelin proteins may confer strong resistance in beans.

1.4 Transgenics

Transgenic technology is a relatively new method of crop protection, which can generate new plants and “super seeds” with value-added traits (Baker and Kramer 1996, Estruch et al. 1997, Gatehouse and Gatehouse 1998). Genes from microorganisms, plants, and animals are being expressed in new varieties that, as a result, are more resistant to insect and fungal pests than the parent variety. These recombinant transgenes encode novel proteins detrimental to critical insect life processes in the endocrine, nervous, skeletal, and digestive systems and include α -amylase inhibitors, digestive system toxins, anti-nutritional proteins, hormone-metabolizing enzymes, neurotoxins, carbohydrate-binding proteins, chitinases, and proteinase inhibitors. With the development of tissue-specific promoter systems, the potential to impact the population growth of stored-product insects that feed on all or only parts of cereals and legumes, i. e., seeds, as well as their products has dramatically improved.

To date there have been only 6 reports of the application of transgenic technology to stored-product insect control. The 1st application was that of Shade et al. (1994) who used a plant protein, an insect digestive enzyme (α -amylase) inhibitor, to prevent insect larvae from digesting their food and obtaining sufficient nutrition from starch. Stored-product insects generally have relatively high levels of amylolytic enzymes in their guts to digest the high levels of starch in their foods. Because the common bean is naturally resistant to bruchid beetles largely due to the presence of one or more α -amylase inhibitors (Suzuki et al. 1993, Grossi de Sa et al. 1997). Shade et al. (1994) and Schroeder et al. (1995) hypothesized that the inhibitor gene would be a good candidate for a genetic engineering approach that would make other seeds (pea, chickpea, cowpea, and adzuki bean) resistant to bruchid infestations. The researchers demonstrated that transgenic pea seeds expressing the α -amylase inhibitor of the common bean were resistant to predation from bruchid beetles. The levels of the amylase-inhibiting protein in the transgenic pea seeds were as high as that in wild-type bean seeds and the peas were resistant to the pea, cowpea, and adzuki seed beetles. Field trials of Australia's pea crop in 1998 showed that these genetically modified peas were 99.5% resistant to bruchid attack and that no insecticides were required during their production or storage (T. J. Higgins, personal communication).

The 2nd application involved testing transgenic corn seeds from a commercial seed company that contained a proteinaceous endotoxin from the soil bacterium *Bacillus thuringiensis* (Bt). The effects of both transgenic and nontransgenic corn seeds on 3 species of stored-product insects were studied (Bh. Subramanyam and L. Locke, unpublished data). The transgenic hybrids contained Bt endotoxin CryIABb, which is a toxin specific for lepidopteran larvae. As expected, little or no effect on the growth and development of 2 coleopteran species, *T. castaneum* and *S. oryzae* occurred on the transgenic corn. There was, however, significantly higher larval mortality of *P. interpunctella* on the Bt seed lots than on the nontransgenic lots.

The results indicated that seeds with high expression of Bt toxins have potential for suppressing Lepidoptera, but not Coleoptera, in stored products. Bt toxins with sufficient efficacy for stored-product beetle control have not yet been identified.

The 3rd application involved the use of an animal protein, the chicken egg white protein avidin, which can exert anti-nutritional lethal effects on insects from many orders (Morgan et al. 1993). Transgenic corn with high expression of chicken avidin had little susceptibility to common insect pests that damage nontransformed corn kernels during storage in the United States (Kramer 1999). Avidin corn was toxic to both internally-feeding (*S. zeamais* and *R. dominica*) and externally-feeding insects (*O. surinamensis*, the depressed flour beetle, *Palorus subdepressus* (Wollaston), and *T. castaneum*). Avidin is a relatively unique biopesticide whose effects can be completely reversed by supplementation with an antidote, biotin. The avidin corn is protected from insect damage until the transgenic protein is denatured by heat treatment. Although toxic to insects, this grain was not toxic to mice, indicating that there may be little or no toxicological problems for humans and animals that consume the avidin-containing transgenic corn (J. Howard, unpublished data).

The 4th application involving transgenic seeds utilized 2 strains of the cabbage seed weevil, *Ceutorhynchus assimilis*, which exhibited differential susceptibility to transgenic oilseed rape expressing oryzacystatin, the cysteine proteinase inhibitor from rice (Girard et al. 1998). Contrary to expectations, one of the strains exhibited an increased growth rate when fed the transgenic seeds, whereas the other strain was unaffected. These results were surprising because inhibition of digestive proteinases *in vitro* by oryzacystatin was observed in both strains. Apparently, biochemical interactions other than those predicted from *in vitro* experiments can take place *in vivo* between the recombinant biopesticide and a nontarget physiology, which may lead to an effect that is beneficial to the insect pest.

A 5th application using transgenes for stored-product insect control involved rice transformed with a gene for a bifunctional enzyme inhibitor from barley, the amylase-subtilisin inhibitor (Ohtsubo and Richardson 1992). The effects of 3 varieties of transgenic rice on *C. pusillus* were examined in a preliminary study (Kramer and others, unpublished data). One transgenic variety caused a 30% higher mortality than the control or 2 other transgenic rice plants. Additional studies are needed to evaluate the potential of using these types of bifunctional enzyme inhibitors as biopesticides for stored-product insect control.

The 6th and last application of transgenic technology to stored-product insect pest control utilized the barley trypsin inhibitor CME expressed in wheat via biolistic bombardment of cultured immature embryos (Altpeter et al. 1999). The survival of early instars of *S. cerealella* on transgenic seeds was reduced by up to 30% relative to untransformed seeds. Expression of the CME protein in transgenic leaves, however, did not affect predation by the leaf-feeding migratory grasshopper *Melanoplus sanguinipes* (F.). Thus, in this case, the barley protein exhibited potential for controlling an important storage pest but not a field pest of grain crops.

Many other transgenes, including immunoglobulins, lectins, chitinases, cholesterol esterases, vegetative insecticidal proteins, and peroxidases, have been expressed in plants (Carozzi and Koziel 1997). Although many of these studies

have demonstrated substantial protection of plants from insect predation, we are unaware of any plans at present to develop such biopesticidal transgenes commercially for stored-product insect control. This lack of effort may be due, in part, to the absence of financial incentives for development of the technology specifically for stored-product insect control. Furthermore, insects are rather flexible and able to adapt to the presence of some of these biopesticidal proteins, allowing the pests to continue feeding and developing as was observed for some proteinase inhibitor transgenes (Girard et al. 1998). Application of transgenic technology for stored-product insect control will probably occur only as an extension of the same for field crop insect control.

2.0 PRESENT USAGE

Currently, resistance to attack by insects during storage is not generally considered during breeding programs or evaluated prior to release of commercial varieties. Nevertheless, recent studies have shown that commercial United States corn hybrids (Throne and others, unpublished data) and oat cultivars (Throne and others., unpublished data) vary greatly in their susceptibility to stored-product insect pests, even though these hybrids and cultivars were not bred for resistance to stored-product insects. For United States wheat and triticale, there currently does not appear to be any germplasm with significant resistance to stored-grain insects. More expansive studies in India have been successful in providing wheat varieties with significant levels of resistance.

New rice varieties have resulted in dramatic increases in yield, but the acreage planted with rice varieties resistant to insects represents only a small fraction of the total rice production. Furthermore, these varieties are bred for resistance to insects affecting crop production such as the brown planthopper, *Nilaparvata lugens* (Stahl) (Heinrichs et al. 1985, Rao et al. 1998, Lee et al. 1999). As of 1985, varieties with resistance to at least 8 pest species that affect production have been released. No breeding lines for insects attacking stored rice, however, were available, and no varieties resistant to stored-grain insects have been released. Transgenic crops currently are not used for control of stored-product insect pests.

2.1 Implementation in IPM and outlook for the future

For United States wheat and triticale, there currently does not appear to be any germplasm with significant resistance to stored-grain insects that can be incorporated into commercial varieties in the near future. Additional work is needed to characterize such germplasm and incorporate these factors into new wheat varieties. Currently, wheat and triticale breeders in the United States do not generally evaluate selections for resistance to stored-grain insects. Successful development of wheat and triticale varietal resistance programs in the United States will require closer cooperation between entomologists and plant breeders. Commercial corn hybrids and oat cultivars that are relatively resistant to storage insects have been identified, but this information is not currently being used in selecting varieties for planting or in breeding programs.

For United States rice varieties, limited screening for resistance against *S. oryzae* and *R. dominica* has been conducted, and any resistance that has been found was not sufficient to prevent infestation (Cogburn 1977a). More varieties have been found with low levels of resistance to *S. cerealella*, but again, the level of resistance is considered too low to prevent significant damage during storage (Russell and Cogburn 1977). Currently, there is no organized program in the United States for evaluating rice germplasm for resistance to storage insect pests, nor is there a breeding program to enhance any available resistance (Dr. Anna McClung, USDA-ARS Rice Research, Beaumont, Texas, personal communication). Nevertheless, despite a lack of current research in this area, varietal resistance in rice has the potential to be an important component of an overall pest management program, especially in view of the loss of alternative control technologies.

2.2 Limitations of resistance mechanisms

Efforts to isolate and develop varietal resistance in grain legumes have been hampered in 3 ways, and these same limitations probably apply to cereal varieties. First, mechanisms of resistance identified thus far show a high degree of target specificity, i.e., traits conferring resistance to one bruchid species have little or no effect on another (Birch et al. 1985). It may not be surprising that factors promoting resistance to Old-World seed beetles would be ineffective against New-world species (Xavier-Filho et al. 1989, Grossi de Sa et al. 1997), but mixed responses have also been observed among closely related species and among geographic populations of the same species (Ofuya and Credland 1995a,b). For example, arcelin proteins toxic to *Zabrotes* have little effect on *Acanthoscelides* (Hartweck et al. 1997), and *C. maculatus* is less sensitive to α -amylase inhibitor from common bean than is *C. chinensis* (Shade et al. 1994). Dick and Credland (1986) detected pre-existing biotypic variation in the response of *C. maculatus* to landrace TVu 2027, i.e., some populations performed better on this host than others even before any population was exposed to the cultivar. Most studies with cereal insect pests have been limited to one or a few species, so we do not know whether varieties with resistance to tested species would also have resistance to other pest species.

A second limitation to breeding for varietal resistance is the rate at which insects evolve to overcome the resistance trait. Populations of *Z. subfasciatus* may have become insensitive to amylase inhibitors by virtue of evolving either proteases that degrade inhibitors or by producing inhibitor-insensitive amylases (Ishimoto and Chrispeels 1996, Giri and Kachole 1998). Widespread, cosmopolitan bruchids, such as *C. maculatus* and *Z. subfasciatus*, have shown rapid adaptation to novel host species and may show similarly quick responses to resistance traits (Desroches et al. 1997). Shade et al. (1996) found that around 50 generations of laboratory selection were sufficient for a *C. maculatus* population to perform as well on TVu 2027 as it did on a susceptible cowpea variety. More importantly, adaptation to TVu 2027 allowed the pest population to develop rapidly on other cultivars with a similar mode of resistance.

Finally, cultivars with strong resistance must also possess acceptable agronomic characteristics. Beyond the usual considerations of yield, germination,

consumer acceptance, resistance to field insects and pathogens, and other breeding objectives, programs incorporating biochemical resistance must consider the effects of each compound on nontarget organisms, including mammalian or human physiology. Many of the seed proteins that confer resistance to bruchids (lectins and protease inhibitors) are heat-labile, which means that any negative effects on human and livestock digestion should be reduced or destroyed by cooking (Marconi et al. 1997). Other compounds, such as the tannins prominent in the testa of dark-seeded varieties, are heat-stable and may interfere with protein digestibility after consumption. Beans and cereals are routinely processed before consumption, so effects of resistance on processing must be considered. For example, because the major resistance base against insect damage in rough rice is the hull, breeding for tighter hulls may be disadvantageous because of adverse effects on the milling process.

A few disadvantages to transgenic technology have been identified. First, there may be less than widespread consumer acceptance of this technology. Whereas consumers in the United States generally are accepting of transgenic crops, European consumers are more vocal in questioning their use (Swoboda 1996, Wood and Fairley 1998). Second, depending on the particular biopesticide, there could be toxicological problems for humans and livestock. This should not be the case with an insect-specific biopesticide such as the Bt toxins, but each biopesticide must be evaluated individually for potential side effects when utilized in foods and feeds. Finally, there is good reason to expect that insects will develop resistance to biopesticides, just as they have developed resistance to most other insecticides (Gould 1998). Thus, management practices must consider this possibility and efforts to prevent or delay resistance, such as including nontransgenic refuges or pyramiding multiple resistance genes and adding other insecticidal proteins with completely different mechanisms of action to the crop, must be undertaken. Whether insect resistance to biopesticides actually occurs will depend on the particular kind of biopesticide and the conditions under which it is used. The effects of incorporated biopesticides on nontarget organisms have generally not been determined, but would normally be expected to be less than with broadcast spraying of conventional insecticides.

2.3 Advantages

The advantages of developing seeds with resistance to insects are many, and the technology is fully compatible with IPM practices. Varietal resistance offers a passive alternative control technology that can significantly suppress pest insect populations and help maintain grain quality during storage. As a passive technology, no input is required from the grower other than varietal selection. Use of resistant varieties should result in reduced applications of conventional pesticides. Treatment efficacy would be independent of environmental effects, whereas insecticides may degrade at high temperatures or humidities or be inactivated by ultraviolet radiation. The control technology would be in place for the entire storage season. Resistance mechanisms or biopesticides can be truly systemic, reaching all parts of the seed that traditional pesticides may not permeate.

The only insects affected usually are those that actually feed on the seed, thus beneficial insects and others that do not feed on the seed generally would not be affected. However, there is evidence for tritrophic effects of a transgenic potato upon an insect predator (Birch et al. 1999).

Transgenic crops have some additional advantages. Cost analyses indicate a clear advantage to transgenic versus nontransgenic crops in terms of costs of production, utilization of traditional pest control methods, management, and yield losses resulting from insect predation. Also, proteinaceous insecticides can be designed to be in place when the insect pest is at its most sensitive stage of its life cycle. The active agents in transgenic seeds are biodegradable proteins, so there is little potential for ground water contamination or other environmental problems.

2.4 Economics

The seed industry in the United States enjoys the status of being one of the few industries that is not regulated. The conventional crops used today for food and feed continue to undergo genetic improvements, and are considered safe and have caused no concern for regulatory intervention. In cases where there have been genetic improvements to insect resistance, there has been no need to register these products. The farmers are given a score relating to insect control, and they can choose which seed they want to plant without any regulatory consequence. This model for insect resistance in the field should serve as the framework for postharvest insect resistance as well.

Several countries outside the United States do require that all new seed products be registered. A new seed product is defined as one that has different characteristics than existing products. This difference can be higher yielding grain production, better resistance to insects, or some other improved agronomic trait. While this is a straightforward process in most countries, it usually requires several years of testing.

Transgenic crops, regardless of the trait, are regulated in the United States as well as in other countries. Many factors are considered before regulatory approval can be obtained. Some factors include source of the gene, open pollinating versus self-pollinating crops, toxicity to other animals, especially humans, and acreage planted. Fortunately, insecticidal proteins, such as Bt proteins, have been incorporated into crops and are currently being used for insect control in the field. This can serve as a model for getting postharvest insect resistance through regulatory approval. As an alternative, transgenic grain can be used to produce an insecticide, and the grain containing the insecticide can itself be used directly and not as a food or feed source. Both of these cases are examined below.

Insect resistance in grain

This is the standard method of using transgenic grains with insect resistance. A grain cultivar that has agronomically desirable traits is genetically modified to include pest resistance. The resulting grain crop is used for food or feed. This has the advantage of providing effortless pest management for the farmer, but it can

increase the regulatory requirements. The producer would need to demonstrate that the gene product is safe. In grain this would require testing to show that the product has no deleterious effects on animals or humans. If the gene product were also expressed in other parts of the plant such as pollen, then studies showing its effect on plant pollinators would also be needed. With today's technologies, gene products introduced into the germplasm are random; therefore, in addition to any direct effects of the introduced gene, it is important to show that there are no major alterations to the other properties of the grain. These studies will likely take between 3-5 years for regulatory approval with a cost in the millions of dollars. While it is unlikely this would be done exclusively for minor crops or minor pests, the results would have a huge impact on the major postharvest pests and the economic gain would more than offset the additional research costs.

Transgenic grain as an insecticide

In this case, the transgenic plant is used to produce grain that contains an insecticide. The harvested grain can then be further formulated or used directly for insect control. For example, grain that contains an insecticidal protein could be ground and mixed with conventional grain in storage. Insects in the stored grain may feed on the transgenic grain and die. The insecticidal grain would be removed during the normal cleaning process before milling. Having the insecticide in the grain poses an advantage in that it may be considered pre-formulated. The regulatory procedure will be similar to that for other insecticides. The type of studies required for insecticides are standard and include toxicity to animals as well as persistence in the environment. The registration time and costs will be dependent on the type of product introduced, whether the gene product is well characterized, and whether the toxicology and mode of action is in the public domain. If a synthetic gene product is made in plants and little is known about its toxicology or environmental impact, this would prolong the time needed for registration. In addition, there would also be additional regulatory requirements to ensure that the crops were not mixed with the food supply. This would require methods for isolating the crop from other food crops and restricting pollination. For self-pollinating crops this would be routine, while open-pollinating crops would require extra handling. The toxicity of the compound to animals would greatly influence the precautions to be taken. Cost of regulatory packages for this type of product could run into millions of dollars.

3.0 RESEARCH NEEDS

One of the major needs in the use of conventional and transgenic resistance for control of storage insect pests is the identification of resistant germplasm and specific mechanisms of resistance to storage pests. Rapid tests for identifying resistance factors would speed development of new varieties. For example, ferulic acid content of corn may be quickly determined and has been correlated with resistance to *S. zeamais* and *P. truncatus* (Arnason et al. 1997). Characterization of resistance levels in currently used commercial varieties may help to identify

mechanisms of resistance that are compatible with agronomic traits and provide an immediate management tool for producers through selection of resistant varieties for planting.

Development of transgenic crops requires screening for proteins and other compounds that are toxic to stored-product insects. Currently, we rely on screening programs targeted for pests of crops in the field. This might include screening cereals for inhibitors of enzymes required for insect digestion and other physiological processes, or screening for naturally occurring substances in the plant or harvested grain that interfere with normal insect development. Genes for toxins produced by plants and other organisms are also potential candidates for incorporation into transgenic crops.

Further development of legume resistance to storage insects will likely involve both increases in constitutive levels of resistant factors (e.g., hypersynthesis of seed proteins) and the introduction of novel characteristics with transgenic methods. Transgenic methods appear promising because of early successes (Shade et al. 1994, Schroeder et al. 1995, Chrispeels et al. 1998) and because some resistance factors are inherited in a simple Mendelian fashion and are thus potentially transferable to cultivars that already have the requisite agronomic characteristics (Young et al. 1992, Dongre et al. 1996). Identifying resistance genes will be aided by the development of linkage maps for legumes; RFLP, AFLP, and RAPD markers have already been used to construct such a map for cowpea (Ménendez et al. 1997). Further research should also clarify some of the confusion surrounding mechanisms of resistance in particular plant-pest associations. The insecticidal actions of lectins, arcelin, amylase inhibitors, and other plant proteins may be better understood once the 3-dimensional structures of these proteins and their complexes with ligands, receptors, and enzymes are known (Fabre et al. 1998).

A final objective should be the integration of plant resistance with other means of controlling storage pests. Plant resistance would appear to be compatible with most cultural and physical control measures that are applied to either large commercial stores or small traditional granaries. These measures include optimal harvest timing, hermetic storage, temperature manipulations, and the use of inert substances (ash, sand, etc.) to cover seeds (van Huis 1991). An especially important consideration for stored-legume IPM is the compatibility of varietal resistance and biological control. For example, egg parasitoids can provide significant control of *C. maculatus* populations in storage, but host-plant variation can affect both the performance and sex ratios of the parasitoid (van Huis and de Rooy 1998).

Studies of tritrophic interactions between seeds, storage pests, and natural enemies are needed. Birch et al. (1999) were the 1st to demonstrate adverse tritrophic interactions involving a transgenic crop. Expression of a lectin gene for insect resistance in a transgenic potato line caused a decrease in fecundity, egg viability, and longevity of a predatory ladybird, *Adalia bipunctata* (L.), feeding on peach-potato aphids, *Myzus persicae* (Sulzer). Possible adverse effects on nontarget species caused by transgenic pollen containing Bt toxins has also been suggested, although further studies did not support this hypothesis (Losey et al. 1999). To develop safe and durable stored-product pest resistant crops, it will be necessary to carefully choose the best anti-pest genes, use precisely targeted gene expression

systems, institute measures to limit potential for development of resistance in the pest population, test for effects on nontarget organisms, and include studies of tritrophic interactions between the plants, target pests, and natural enemies under both laboratory and field conditions.

Perhaps one of the best ways to promote development and use of varietal resistance in stored-products is to relate economic losses to the variety that was planted. Producers buy seed based mostly on yield performance. The potential losses in storage are not considered when choosing a variety for planting. This practice results in postharvest pests being overlooked when breeders and genetic engineers develop new varieties. Data on storage performances of currently used and new varieties need to be obtained and made available to producers. This would eventually result in producers using both field and storage performances as criteria in selecting varieties for planting. Breeders and genetic engineers will respond to this need by developing varieties that perform well in the field and in storage.

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