INHERITANCE OF STEM BORER RESISTANCE IN MAIZE (*ZEA MAYS* L.)

By

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Chapter I

1-1 General Introduction

Maize (*Zea mays* L) is regarded as the most important cereal crop valued as a human food source. The average yield for industrial countries is 6.5t/ha, compared with only 2.5t/ha for developing countries (Dowswell *et al.*, 1996).

Dowswell *et al.* (1996), stated that the maize yield is 2.0t/ha in South Africa, 1.5t/ha in India and 1.7t/ha in Kenya, which is low compared to 7.5t/ha, 7.1t/ha, and 7.8t/ha in the United State of America, France, and Italy respectively.

Nevertheless, 64% of the world's maize area is found in developing countries that harvest only 43% of the world production (Dowswell *et al.*, 1996). Most of the maize in Africa is grown by subsistence farmers and yields are generally low, with averages less than half that of Asian and Latin American yields (Polaszek & Khan, 1998). Sub-Saharan African countries such as Kenya, South Africa, Tanzania, Ethiopia and Nigeria are principal producers of maize. South Africa is the only one of these exporting maize (Polaszek & Khan, 1998).

Maize has been put to a wider range of uses than any other cereal as a human food, as a feed-grain, a fodder crop, and for hundreds of industrial purposes because of its broad global distribution, its low price

relative to other cereals, its diverse grain types, and its wide range of biological and industrial properties.

More than half of all maize is utilized directly as human food in the Andean countries of South America, Mexico, Central America, and the Caribbean. In Africa and Southeast Asia it accounts for at least 15% of the total daily calories in the diets of people in 23 developing countries, nearly all in Africa and Latin America.

From 1950 to 1980 world maize production increased from about 145 million tons to 450 million tons, growing at a faster rate than either wheat or rice (Dowswell *et al.,* 1996). In the industrialized countries, more than 90% of the growth in maize production can be attributed to the adoption of yield-increasing technologies. In the developing countries, area expansion has accounted for about half of the growth in the maize production, but yield-increasing technologies are becoming more important. Maize producers worldwide attempt to solve the question of yield losses caused by diseases, insects and other causes of damage through the development of resistant varieties. Studies on insect resistance, mainly of stalk borers, began in the USA and Europe, studying European corn borer, *Ostrinia nubilalis*, which is considered the most important. It causes severe damage estimated at hundreds of millions of US dollars in crop losses in the United States and Europe.

O. nubilalis is distributed through North America, Europe, the Middle East, and North Africa (Ortega *et al.,* 1980). Techniques have been developed for artificial infestation of plants with European corn borer. As

a result more information is available on *O. nubilalis* resistance than is true of any other insect pest.

In Africa it is of great importance to study all insects that cause significant plant damage such as *Sesamia calamistis*, (Hampson) *Chilo partellus* (Swinhoe), *Busseola fusca* (Fuller), and *Eldana sacharina* (Walker). These are considered the most important stem borers of maize on the continent (Bosque-Perez & Mareck, 1990; Van Rensburg & Malan, 1990). Unfortunately, in most African countries, breeding for resistance has, to date, received relatively little or no attention.

The maize stalk borer *B. fusca*, is a major pest requiring the application of expensive chemical control measures in order to avoid severe crop losses (Seshu Reddy, 1985; Kaufmann, 1983a; Egwuatu & Ita, 1982; Walker, 1960; Ogunwolu *et al*, 1981). In most developing countries the principal producers are still the peasants and small-scale farmers, who frequently do not have access to chemical means of controlling insects and who frequently cannot afford chemical control, even when it is available. An economical solution to this pest problem is to breed for resistance against *B. fusca* and other insects causing damage (Zavaleta & Kogan, 1984). The maize stalk borer is generally considered the most widely spread and most destructive of all insects attacking maize in South Africa (Smithers, 1960; Rose, 1962; Walters, 1975; Van Rensburg *et al.,* 1978). It has for many years been known as a major pest of maize, causing an estimated annual loss of 10% of the total maize production (Mally, 1920).

Since 1950, maize has become one of the most important agricultural crops in South Africa, with production exceeding 10 million tons in favorable years. Bearing in mind that production costs maintain a steady increase, it is evident that much more effort must be put into breeding for resistance for maize pests (Van Rensburg, 1982).

The use of resistant crops is now recognized as a useful method of controlling pests and diseases, and therefore various attempts have been made to develop resistant maize cultivars as an alternative or addition to chemical control. The goal of incorporating resistance into commercial hybrids has been a common task of most breeders in the world in recent years. In this way many breeders have shown that both additive and non-additive gene effects are important for inheritance of maize resistance to all three parameters of stalk borer damage (Guthrie, 1987a; Ajala, 1992; Van Rensburg & Van den Berg, 1995; Pathak & Othieno, 1990, 1992).

Recurrent selection is considered to be of use to accumulate the genes responsible for stalk borer resistance in breeding programs. It is known that resistance to stalk borers, mainly to second generation *O. nubilalis*, (Hubner), is controlled by at least five genes (Onukogu *et al.*, 1978; Guthrie, 1987a; Schon *et al.,* 1993) stated that the studies done during the past several decades on leaf feeding resistance in maize (first generation *O. nubilalis*), and to sheath-collar feeding by second-generation *O. nubilalis*, indicate that various resistant inbreds may carry several factors conditioning resistance.

Assuming that these factors and alleles are completely independent in their performance relative to resistance, backcross breeding would not normally be considered a practical approach for developing plants with improved resistance (Sarjes *et al.*, 1994).

It was found by Guthrie (1987b) that leaf feeding by first-generation, *O. nubilalis* (Hubner) is conditioned by at least eight genes. Reciprocal translocation studies showed that at least 12 of the possible 20 chromosome arms contribute a minimum of 12 resistance genes to the two European corn borer generations. This number of genes rules out the possibility of using a backcross procedure to transfer resistance to susceptible maize genotypes. The use of molecular probes to track movement of both favorable resistant alleles and recurrent parent alleles, increases the feasibility of backcross breeding for complexly inherited traits.

Sax (1923) was the first to show that quantitative trait loci (QTL) could be associated with marker loci in crosses between inbred lines. Today, with rapid advancement of molecular technology, it is possible to use molecular marker information to map a major part of quantitative trait loci (QTLs) on chromosomes (e.g., Paterson *et al.,* 1988, 1991; Stuber *et al.,* 1992).

Genetic diversity, selection response, and the analysis of quantitative trait expression are issues of importance and interest to all plant breeders. Maize is an excellent species for QTL analysis. Despite of assumptions made by many scientists that the additive and non-additive genes are both important for inheritance of stalk borer resistance in

maize, there has been limited success in transferring genetic resistance into agronomically desirable cultivars due to insufficient knowledge about how the resistance is inherited.

Objectives of this study were to:

(i) Determine inheritance of resistance to the stalk borer *B. fusca*, following the phenotypic and genetic expression of the resistance based on artificial infestation with first instar larvae.

(ii) Identification and characterization of the genetic factors contributing to resistance against *B.fusca* through antibiosis assessment.

(iii) Screen linkages to insect resistant genes in the F2:3 population (from resistant x susceptible crosses) using AFLP markers.

(vi) Estimate the contribution of AFLP markers to the breeding program for insect resistance against *B. fusca*.

CHAPTER 2

LITERATURE REVIEW

2.1 - Insect plant relationships

In recent decades, there have been substantial changes in crop production practices. Nevertheless, the maize crop remains subject to attack by a complex of insects from the time it is planted until it is utilized as food or feed.

Other crops, particularly small grains, forage grasses and legumes provide sources of insects that attack maize. This ecological relationship is a part of the maize insect problem and from this stems a need to understand the dynamics of this problem.

The most important maize stalk borers are: The European corn borer *O. nubilalis* (Hübner) in North America, Europe, Middle-East, and North Africa; the Asian corn borer *O. furnacalis* (Hübner), in Asia and in the Philipines; the spotted stem borer *Chilo partellus* (Swinhoe), in Asia and Africa; the Asiatic rice borer *C. suppressallis* (Walker), in Asia; the Oriental maize borer *C. agamemnon* (Bles); the African maize borer *Sesamia calamistis* (Hmps) in Africa; the pink stem borer *S. inferens* (Walker) in Asia; the African maize stalk borer *Busseola fusca* (Fuller) and the African sugarcaneborer *Eldana sacharina* (Walker) in Africa; the American sugarcane borer *Diatraea saccharalis* (Fabricius) in the Americas; the Southwestern corn borer *D. grandiosella* (Dyar) in

southern USA and Mexico; the Fall armyworm *Spodoptera frugiperda* (J.E Smith) in Southern USA and Latin America and the African Armyworm *S. exempta* (Walker) throughout Africa south of the Sahara. This study deals with *B. fusca* (Fuller) (Leptodoptera: Noctuidae) that is considered one of the most important borers attacking maize in the region South of the Sahara. It is of a great economic importance for maize production in South Africa. The *B. fusca* interaction with the maize crop is basically the same as recorded for other lepidopterous borers.

The first generation infestation develops from moths emerging in spring (October) from diapause larvae overwintering in maize stalks. The moths are attracted over great distances to young maize plantings, where they oviposit underneath the leaf sheaths (Mally, 1920). According to Van Rensburg & van den Berg (1995) egg-laying by *B. fusca* takes place from three to six weeks after crop emergence. However, some egg-laying can take place later than six weeks after plant emergence if moths do not have a choice of younger plants (in case of plantings later than mid-November).

The number of eggs per batch varies from five to 37 (Van Rensburg & Van den Berg, 1995), with the majority of egg batches (79%) containing 11 to 25 eggs, considerably fewer than the maximum number of 300 recorded by Kaufmann (1983b) in Nigeria, but comparable to the average of 22.1 eggs/batch found by Van Rensburg (1981) in South Africa and 25.2 eggs/batch recorded by Usua (1968) in Nigeria.

However, the result of other workers diverge; Harris (1962) gives corresponding figures of 30-100 and 1000 per female, while Ingram (1958) found an average of 70 per batch and a maximum of 568 per female. Van Rensburg *et al.* (1987) in South Africa stated that, accepting 203.4 as the average number of eggs per female, the implication is that seven to eight egg-batches are produced and that a single female can infest several plants. This corresponds with the eight batches per female found by Ingram (1958). The position in which the eggs are laid is correlated with the time of egg-laying. From one to three generations occur annually, dependent on temperature. The majority of the larvae enter diapause in the autumn, spending the winter months in the plant stems, generally in the part just below ground level.

Larvae feed successively on developing leaf tissue, tassel glumes, stalks, and finally stem tissue. Van Rensburg *et al.* (1987) found that larvae feed mainly in the whorls of plants until the fourth instar. Most larvae enter the stem as soon as the tassel emerges. The larvae are therefore exposed to a variety of food sources, each of which probably has a different nutritional status and therefore has a different effect on larval development. The larvae pupate in the stem after chewing a small-perforated "window" in the outer stem tissue, which is pushed out later by the emerging moth.

In lower latitudes diapause of *B. fusca* may occur twice, in winter and in the dry season (April-October), as mature larvae inside dry stalks (Kfir, 1988). Many larvae rest in the lower part of the stalks beneath the soil surface, where they are protected from natural enemies and are well insulated against adverse climatic conditions. Diapause by *B. fusca* in

the dry season was reported from several countries in Africa (Mally, 1920; Ingram, 1958; Smithers, 1960; Harris, 1962; Usua, 1970; Kfir, 1988).

Taking into consideration this kind of behavior of *B. fusca* in its relationship with maize, the effort made by maize producers to avoid losses caused by *B. fusca* have been going in different directions such as using chemical treatment and biological and cultural procedures.

No doubt the cumulative effect of parasitoids, microbial pathogens and predators curtail populations of *B. fusca* and *C. partellus*. However, their activity is not enough to reduce the pest populations to below an economic damage level. An economical solution to the problem is to breed for resistance against *B. fusca* and *C. partellus* (Zavaleta & Kogan, 1984).

2. 2- Breeding for insect resistance.

Farm crops are cultivated for grain, forage, fiber, oil and other products of commercial importance. Their yield and the excellence of their market quality or their nutritional value are of direct concern to farmers. From sales and from their use as feed they compensate for labor and investment in their production. To increase his profits the farmer is constantly searching for more efficient procedures to increase production and to improve their markets or nutritional value of the crop.

The potential productivity of the plant has traditionally been increased by modifying its morphological characteristics such as the mass of

individual seeds or by modifying physiological traits such as harvest index, the utilization of nutrients, or tolerance to stress. Breeders strive for early maturity, increased resistance to heat, drought, disease and insect damage. Host plant resistance is an important component of integrated pest management of maize. Breeding for insect resistance frequently incorporates various conventional breeding techniques. Often the breeding method of choice is a form of recurrent selection. Using recurrent selection, the selected resistant progenies are intercrossed to increase the frequency of favorable resistant alleles.

Barry *et al.* (1983, 1984, 1985) and Klenke *et al.* (1986) reported successful use of recurrent selection to produce improved sources of resistance to *O. nubilalis*. Various modifications of the pedigree breeding system also have been used to develop *O. nubilalis* resistant lines and hybrids. Guthrie *et al.* (1985) reported success using pedigree breeding to develop inbred line B86. Using the same method, the resistance source DE811 was developed by Hawk (1985). There are many effective conventional breeding methods that may be used to improve resistance to insect pests depending on the source of resistance and the goal of the breeding program.

Sargers *et al.* (1994) proposed a general pedigree breeding procedure for developing lines with improved levels of *O. nubilalis* resistance as shown in Table 1. Although substantial gains were made using conventional breeding methodology, additional methods of improvement became necessary in order to develop better sources of resistance.

Significant progress in this direction has to date been made largely due to the efforts of individuals, using conventional methods which proved that effective insect resistance in maize is available and with enough effort the trait can be transferred to various genotypes of maize. Studies on insect resistant maize began in the early 1900's when Hinds (1914) demonstrated the value of maize husk tightness or thickness for corn earworm *Helicoverpa zea* (Boddie) resistance.

Resistance is a relative property, based on the comparative reaction of resistant and susceptible plants, grown under similar conditions, to the pest insect. Resistance may be due to the presence of olfactory repellents, feeding or oviposition deterrents, toxins and the absence of feeding or oviposition stimulants. In one instance, lack of nutrients has been shown to affect insect resistance in maize (Smith, 1994).

Penny *et al.* (1967) determined that maize resistant to *O. nubilalis* larvae had an ascorbic acid content that was inadequate to support normal growth of larvae.

Resistance may also be the result of the density of external or internal plant structural features that either alter insect behavior or reduce insect digestion. In some maize varieties the content of silica containing cells is high enough to adversely affect *O. nubilalis* larval feeding and impart some resistance (Rajanridpiched *et al.,* 1984).

Smith (1994) defined plant insect resistance as the genetically inherited qualities that result in a plant of one variety or species being less

Table 1 -General pedigree procedure for developing lines with improved levels of *O. nubilalis* resistance.

(YEAR1)

Susceptible inbred x Resistant inbred

↓

Self F1

↓

Self F2

↓

(YEAR2)

Screen 150, + F3 progenies self poll

& Select top 10-15%.

↓

Test Cross F4

↓

(YEAR 3)

1-screen F4 hybrids

2- Yield test F5 Tc Hybri

3- Self & select top 10-20%

↓

(YEAR 4)

1- Screen F6 progeny

2- Yield test F5 Tc hybrids

3-Screen F5 Tc hybrids

4- Self & select top 10 - 20%.

↓

(YEAR 5)

F8 repeat

Winter nursery

↓

(YEAR6)

F10 repeat ----------Expand yield testing

↓

↓ ↓

Expand yield testing Expand yield / performance

With more testers Testing

↓ ↓

(Nursery) **YEAR 7-10** –Commercial hybrid

↓ available with improved R.

Breeder seed

↓

Foundation seed **(YEAR 7)**

↓

Improved resistant line

Available **(YEAR 7 - 8).**

damaged than a susceptible plant lacking these qualities. Thus the ecological and structural relationship among plants and insects allow the existence of different types of resistance. So "pseudo" or "false resistance" may occur in susceptible plants due to earlier than normal planting, low levels of insect infestation, or variations in temperature, day length, soil chemistry and plant or soil water content.

"Associational" resistance, refers to a normally susceptible plant growing in association with a resistant plant, and deriving protection from insect predation. "Induced resistance" is the enhancement of a plant's pest defense system in response to external physical or chemical stimuli (Kogan & Paxton, 1983). This occurs in many crops due to the elicitation of endogenous plant metabolites (Pearce *et al.*, 1991).

In addition to the types of resistance described above, three categories have been referred to since their description by Painter (1951):

- Non-preference resistance: the reaction of an insect to a plant.
- Tolerance resistance: describes the reaction of a plant to insect infestation and damage.
- Antibiosis: this is the most evident, desirable and long lasting mechanism of resistance which has been considered for stem borer resistance in maize. In this kind of resistance the biology of the pest insect is adversely affected after feeding on the plant.

Walter (1957) was one of the first to demonstrate that the resistance in silks of some maize lines was due to antibiosis. Straub & Fairchild (1970) and Wiseman *et al.* (1976 and 1981a) showed that silks of Zapalote Chico possessed a *Helicoverpa zea* larval growth inhibitor.

Wiseman & Isenhour (1990) found additional adverse biological characteristics associated with the antibiotic response when *H. zea* were fed on resistant silk-diets (such as prolonged development time, reduced mass of pupae, and fecundity reduced as much as 65% over generations). Wiseman *et al.* (1992a) found significant relationships in four separate tests between reduced growth of *H. zea* and increased maysin concentration, when maysin was fed as a silk diet. Recently two additional cultivars GT114 and PI340856, (Wilson *et al.,* 1991) have been identified with high levels of maysin (Wilson & Wiseman, 1988; Wiseman & Widstrom, 1992; Wiseman *et al.,* 1992a,b). PI340856 has some of the highest levels of maysin found to date, and is highly resistant, while the resistance of PI340853 is high, but the silks do not contain maysin (Wiseman *et al.*, 1992b). The resistance of PI340856 is governed by a single dominant gene (Wiseman & Bondari, 1995), whereas the inheritance of PI340853 silk resistance is not known to date.

Antibiosis has been evaluated on the basis of larval survival by Pant *et al.* (1961), Kalode & Pant (1966), Mathur & Jain (1972), Lal & Pant (1980) and Van Rensburg & Malan (1990). Antibiosis to *Spodoptera frugiperda*, was discovered in the whorl-stage by (Wiseman *et al.* 1981b). They found that *S. frugiperda* larvae fed on resistant genotypes were significantly smaller than those fed on susceptible maize

genotypes, and the consumption of leaves of resistant plants was also significantly less than consumption on more susceptible plants.

Sharma & Chatterji (1971b), Sekhon & Sajjan (1987) and Durbey & Sarup (1984) evaluated different populations and hybrids. In addition to larval survival they studied the antibiotic effect of this germplasm on other biological parameters, namely larval and pupal mass, larval and pupal period, pupal survival fecundity, egg viability, sex ratio and multiplication rate. They reported that the resistant varieties Antigua Gr.1, A x Antigua Gr.1, Antigua Compuesto, Ganga5, J22, J605 and Mex.17 reduced larval survival, larval mass and pupal mass. They also prolonged larval and pupal period as compared to the susceptible local variety Basi.

Williams *et al.* (1983) reported that *D. grandiosella* larvae reared for seven days on callus of resistant maize genotypes were significantly smaller than when reared on callus from susceptible maize genotypes.

Williams & Davis (1987) also reported that *D. grandiosella* and *O. nubilaris* larvae reared for seven days on callus initiated from resistant maize hybrids weighed significantly less than those reared on callus from susceptible hybrids.

Some researchers studied the ingestion, digestion, and assimilation of plant tissue by larvae to determine how the resistant plant affects metabolism. Kumar (1993) and Ng *et al.* (1993) used a gravimetric method to calculate approximate digestibility (AD) and efficiency of

conversion of digested food (ECD) by *D. grandiosella*, *C. partellus* and *B. fusca*.

It was shown that the Mississipi inbreds, particularly, appear to offer great promise from an antibiosis viewpoint. They have some resistance to *B. fusca* and it is possible that further sources of resistance can be obtained from the gene pool with known resistance to *D. grandiossella* and *S. frugiperda*. Genotypes with DIMBOA related resistance seem to be less promising as sources of resistance to *B. fusca,* (Van Rensburg & Malan, 1990). Antibiosis concerns the four different parts of the plant such as stem, whorl, ear and tassel (Chatterji *et al.,* 1971). The cumulative or additive effect of antibiosis in maize germplasm on *C. partellus* and *B. fusca* reared continuously on a particular variety for more than one generation is of a practical significance.

Antixenosis is a new and appropriate term proposed by Kogan & Ortman (1978) to replace Painter's form "non-preference". Antixenosis, or non-preference, denotes the plant characteristics and insect responses that lead to avoidance of a particular plant or variety, for ovipositon, food or shelter or a combination of the three. Differential preference by *C. partellus* in maize has been reported by Singh (1967), Sharma & Chatterji (1971a), Lal & Pant (1980) and Sekhon & Sajjan (1985); while host plant preference by maize stalk borer, *B. fusca*, was reported by Van Rensburg & Van den Berg (1990). They stated that *B. fusca* could, until now, only maintain high populations in areas of intensive host plant cultivation where crop residues abound, in which diapausing larvae can survive adverse conditions.

Ovipositional non-preference against Antigua 2D-118 by *H. zea* was reported by Widstrom *et al.* (1979). *H. zea* moths preferred to oviposit on the adaxial as compared to abaxial surface of young maize leaves of both resistant and susceptible genotypes. Antigua 2D-118, which is less pubescent than Cacahuacintle crosses, was less preferred than Cacahuacintle crosses.

Non-preference by *H. zea* larvae for silks of resistant maize was reported by Wiseman *et al.* (1983a) while non-preference by fall armyworm has been studied using both leaves and silks of the maize plant. Wiseman *at al.* (1983b), found that significantly more fall armyworm larvae crawled off resistant plants than off susceptible plants in the whorl stage.

Different techniques for measuring non-preference in resistant maize were reported by Khan (1994). He stated that non-preference denotes the presence of morphological and/or chemical plant insect behaviour. Techniques for measuring non-preference were presented as follows:

1- Larval orientation and settling: where the female moths are usually responsible for selecting the plants for their larvae or progeny to feed upon. However, upon emergence the larvae must find a suitable site to initiate feeding. The larvae do have the option of accepting the plant as a host or not. Orientation and settling responses of an insect to a plant are generally measured in choice tests by observing which initially orient toward a plant (orientation), and then remain settled for some time for feeding or oviposition.

2- Attraction test: is a method used by Saxena (1990) to determine the attraction of larvae of *C. partellus* to various susceptible and resistant sorghum cultivars and can also be used with maize.

3- Olfactometer: the orientational responses of neonate larvae to the odor of plants, can be studied using various kinds of olfactometers. A Y-shaped olfactometer, used by Chang *et al.* (1985) for *S. frugiperda*, can be used for studying orientational responses of maize stem borers.

4- Choice test: this test was used by Davis *et al.* (1989) for determining the presence of non-preference mechanisms in selected maize hybrids to *D. grandiosella*, and *O. nubilalis*. To determine whether neonate larvae of stem borers orient and settle preferentially on callus initiated from susceptible or resistant plants, larval orientation and settling responses were measured following the methodology of Williams *et al.* (1987). They reported that significantly more *D. grandiosella, D. saccharalis*, and *O. nubilalis* larvae preferred the callus originating from maize hybrids which were susceptible to leaf feeding.

5- Arrest and dispersal: the settling response of lepidopterous larvae to different cultivars can be compared with respect to their arrest and dispersal on plant or plant parts. Robinson *et al.* (1978) reported that more larvae consistently settled on the susceptible inbred WF9 than on the highly resistant inbred CI31A. Kumar *et al.* (1993) using similar methodology, studied larval arrestment of *C. partellus* on three-week-old plants of susceptible and resistant maize cultivars. The mean numbers of larvae recorded from resistant genotypes Mp704 and Poza Rica

7832 was significantly lower than the number recorded from the susceptible control. Ampofo (1986) studied the arrestment and dispersal of *C. partellus* larvae on susceptible and resistant maize plants in field plots.

6- Feeding: this technique records subtle changes in insect feeding behavior on susceptible and resistant plants and can be useful in identification of resistant germplasm. Such changes in insect feeding behavior can be determined either through the measurement of damaged plant parts, or in terms of the amount of food digested. In a no-choice feeding bioassay, Saxena (1990) offered a 7cm long basal segment of a leaf whorl to 20 neonate *C. partellus* larvae, or an internode segment of a stem to a single fourth instar *C. partellus* larva in a glass vial. Kumar *et al.* (1993) used a photometric device (leaf area meter) for measuring area of leaves before and after insect feeding.

7- Oviposition: for most stem borers and other lepidopterous pests, only the adult female has a large and direct influence on host preference. Saxena (1990) developed and used a three-compartment chamber to evaluate the ovipositional response of *C. partellus* under field conditions. Ovipositional preference of stem borer adults to susceptible and resistant maize cultivars can be measured in two-choice tests following the method of Ng *et al.* (1990), Kumar (1992) and Kumar *et al.* (1993) or in a multiple choice bioassay as described by Ampofo *et al.* (1986). Ovipositional response in a no-choice bioassay can be tested following the methodology of Ampofo (1986).

"Tolerance" is the ability of the host plant to support a certain population level of insects due to plant vigour, or the ability to repair the damaged tissue without loss of quality or yield. This mechanism of resistance may be rendered ineffective, however, if the pest population is too large. Tolerance resistance is associated with the plant's ability to recover and yield satisfactorily, despite insect damage. Tolerance also can mean that the resistant plant simply tolerates the pest insect in the presence of a population of insects equal to that which damages a susceptible plant or cultivar (Wiseman, 1994).

In spite of many theoretical disadvantages of breeding for tolerance, great economic benefits have resulted from the widespread use of virus tolerant varieties in more than 20 crop species. Tolerance to attack by many insect pests has also been exploited successfully. In 1972 Wiseman *et al.* reported that, when plants were planted early in the growing season two resistant maize hybrids, Dixie 18 and 471-u6 x 81-1 supported a number of *H. zea* larvae on the ear that were similar to those on the ear of susceptible hybrids, but suffered much less damage.

At a later planting date, the number of corn earworm larvae in the ears of a resistant hybrid was greater, yet the damage to the ears was significantly less than that on the susceptible hybrids. Thus the resistance of Dixie 18 and 471-u6 x 81-1 was identified as tolerance. Ears of tolerant maize hybrids were described by Wiseman *et al.* (1977) as having tight husks, long silk channels, and large amounts of silk that maintain high moisture content over the period of development of corn earworm larvae. In addition, these tolerant hybrids or cultivars were found to have little or no maysin content (Waiss *et al.,* 1979). Later this

was found to be a major factor for the basis of antibiosis resistance (Wiseman *et al.*,1992a,b).

According to Kumar (1994a) maize resistance to *C. partellus* has not been studied adequately, although he considers tolerance as one of the most desirable type of resistance in plants. Using regression of grain yield reduction on foliar damage ratings due to *C. pattellus*, Ampofo (1986) demonstrated the presence of tolerance in resistant genotypes ICZ1-CM and ICZ2-CM. Kumar (1994b) used regression of functional plant loss index (FPLI) on leaf feeding damage by *C. partellus* to elucidate the presence of tolerance in maize genotypes, ER-29SVR, MBR8637 and Poza Rica 7832.

Tolerance can occur in combination with the two other mechanisms, antibiosis and non-preference. Because of its unique nature in plant resistance to insects, the quantitative measurement of tolerance is accomplished by using entirely different experimental procedures from those used to study antibiosis or non-preference. The study of tolerance usually involves comparing yield or plant growth characters (e. g. height) among genotypes by using infested and uninfested plots (Chiang & Holdaway, 1965).

2.3 - Sources of resistance

The first requirement of any program of breeding for resistance must be the finding of a usable source of resistance. Such sources may be present in existing or old varieties, in wild forms of the same species, in closely related species, or even in different genera. The first of these

possible sources is the most useful because there should be no problems of infertility such as that which occurs in interspecific hybrids, and agronomically undesirable characters derived from plants or another species do not have to be bred out (Williams & Davis, 1994).

In maize it was found that when sources of resistance were taken from wild species of maize such as teosinte (*Zea diploperennis*) that have poor agricultural performance, the transfer of this kind of resistance to other varieties with better agricultural features was difficult. Nevertheless, many research organizations and breeders have done considerable research on finding better sources of insect resistance. Genetic variability exists within the maize genome for borer resistance. Pioneering work was done at Mississipi State University, using Antigua and U.S. germplasm. CIMMYT studies supported the origin of a generalized resistance to borers in Antigua germplasm, and suggested that important chromosomal regions controlling this resistance are located on chromosomes 1(L, long arm), 2,3 (L), 5 (L), 10(L), and 9 (S, short arm) (Dowswell *et al.,* 1996).

Thus, through the effort of an international working group of scientists, maize genotypes developed primarily from the Antigua group 2 gene bank and selected from it at CIMMYT have been shown to be resistant to many of the major lepidopterous pests of maize in Africa, Asia, Latin America and North America (Ampofo *et al.,* 1986; Dabrowski, 1990; Dabrowski & Nyangiri, 1983; Davis & Williams, 1986; Davis *et al.,* 1988; Mihm, 1985; Smith, *et al.,* 1989).

In the mid–1980s, research was intensified also by Embrapa / CNPMS, with a large amount of indigenous and exotic germplasm and elite lines tested for resistance to *S. frugiperda*, and *E. lignosellus*. The screening work identified several sources of resistance to these insect pests (Viana, 1992a; 1992b).

Joint breeding efforts of the French National Institute of Agricultural Research and the Center for International Cooperation in Agricultural Research for Development (INRA-CIRAD), France, is directed toward research for well adapted maize populations with effective levels of resistance to leaf-feeding by *S. frugiperda*, one of the main pest constraints in the Caribbean. Caribbean maize has long been recognized as important breeding material for lowland tropics and as a source of resistance to insects. Several populations and inbreds, derived from Caribbean genetic germplasm with resistance to *S. frugiperda* have been identified (Widstrom *et al.,* 1972; Wiseman & Davis, 1979; Scott & Davis, 1981b).

Some varietal resistance against first-generation *O. nubilalis* was identified (Patch & Everly, 1948), but germplasm for second-generation *O. nubilalis* was not readily available in cornbelt germplasm, and labor required for identification prevented screening many germplasm sources.

The Iowa State team of entomologists and breeders has successfully developed inbreds such as B52 and B86, and other germplasm sources with second-generation *O. nubilalis* resistance. In 1975 a new team, including the disciplines of entomology, plant pathology and breeding

was organized in Missouri. In Colombia, this team could work with longer-season maize germplasm, including some tropical material, which could not be done in Iowa. Because second-generation *O. nubilalis* resistant germplasm was not readily identified in the corn belt, it appeared that the logical place to seek new sources of resistance was in maize populations developed by Dr M.S. Zuber, a USDA-ARS maize breeder at the University of Missouri, which he called PR-Mo2, PR-Mo2 x MoSQA and PR-Mo2 x MoSQB. New sources of insect resistance in Europe and America were identified using artificial infestation of plants with insects.

Since 1989, a wide diversity of germplasm has been screened for reaction to natural or artificial infestation by *S. frugiperda* and *H. zea* using the artificial infestation method developed by Mihm (1983a). Previous host plant resistance results demonstrated that controlled, uniform, artificial infestations are needed to develop insect resistant germplasm.

In Africa, based on observations under conditions of natural infestation, significant differences in susceptibility to *B. fusca* among maize genotypes were reported by Kuhn (1979) and Barrow (1985). Van Rensburg & Malan (1990) found pronounced levels of antibiosis to *B. fusca* in maize lines developed in Mississippi for resistance to *S. frugiperda* and *D. grandiosella*. High levels of resistance to *B. fusca* were observed in the Mississipi inbreds Mp705, Mp706, and Mp707 (Van Rensburg & Malan, 1990). New sources of resistance have since been obtained in breeding material developed by CIMMYT, of which CML139 (yellow kernel type) and CML123 (white) proved to be particularly

promising (Van Rensburg & Van den Berg, 1995). This was regarded as a major finding in view of previous investigations that indicated various genotypes with resistance to *O. nubilalis* to be susceptible to *B. fusca*. Resistance to *C. partellus* was reported by CIMMYT and ICIPE (Dabrowski and Nyangiri, 1983). A project on screening for maize resistance to stem borers was started by the International Centre of Insect Physiology and Ecology (ICIPE) in 1979. The research work was concentrated at the Mbita Point Field Station mainly on: (1) maize screening for resistance to *Chilo, Eldana, Sesamia* and *Busseola*; (2) effect of resistant and susceptible cultivars on larval and adult behaviour, development and survival; and (3) mechanisms of resistance in new selected resistant lines (Dabrowski, 1979).

First generation resistance to *D. grandiosella* appears to impart a level of resistance to other borers, such as *O. nubilalis*, *O. furnacalis*, *D. saccharalis, Chilo Spp., Busseola Spp.,* and *Sesamia Spp.*, as well as to *S. frugiperda.*

The IITA developed populations TZBR-Sesamia–1 and TZBR– Sesamia–3 which proved to be good sources of resistance to *S. calamistis* (Mareck *et al.,* 1989). Two other populations, TZBR-Eldana-1 and TZBR-Eldana-2, are the best sources of resistance to *E. saccharina* (Bosque-Perez & Mareck, 1990).

Unfortunately screening for resistance or preliminary field resistance is not so easy as would be expected. The following factors complicate efficient selection: fluctuation of pest populations during the growing season; the unequalities of insect numbers spread over the field and the

occurrence of different insect species. Significant progress in screening for resistance may be achieved when artificial infestation of plants is available (Ortega *et al.*, 1980).

2.4 - Artificial infestation

One of the most important basic components necessary to identify or develop maize germplasm that has host plant resistance to an insect pest, is the capability to efficiently mass culture the species of importance (Mihm 1982, 1983a,b). There is a need for breeders to develop different forms of mass rearing systems. In order to efficiently mass rear a species in addition to a thorough knowledge of the biology of that insect in all its life stages, the followings components are required:

1) A rearing facility, 2) sufficiently trained personnel, 3) natural, meridic, or defined diets, 4) containers and rearing procedures, 5) sources of the pest species to establish a colony.

The main reason for mass rearing of insects in all known different insect rearing programs is to use them in host plant resistance screening or breeding procedures. The insects produced would exhibit the vigour and vitality of the demanding pest population within the geographical and ecological areas that are affected. The maintenance of healthy colonies of insects is to be done under artificial conditions that demand special environmental and sanitation observance, such as variation of temperature during different growth stages of the insect as well as the dark photoperiod (according to the biology of the insect), and regulated humidity.

2.5 - Inheritance of resistance

A better understanding of how resistance to insect attack is inherited becomes of great interest for breeders once a source of resistance is available, in order to develop an efficient breeding program. The resistance gene may interact, the interaction being complementary where two or more non-allelic resistance genes are required to confer resistance. Alternatively, a resistance gene may require the presence of another gene before it can be fully expressed (modifying action). One gene can also mask the action of another. Effects of resistance genes may be additive, for example when the expression of resistance is increased in the presence of two or more different resistance genes. Alternatively one gene may dominate over another non-allelic gene (epistasis). Different genes can control the same resistance mechanism, the presence of any one of these duplicate genes conferring the same level of resistance as any combination of the others (Russel *et al.,* 1974). Knowledge of the interactions between resistance genes can sometimes help the breeder to conduct his program of breeding for resistance less empirically and therefore more efficiently. A great contribution on this issue was made by Mather (1958), Hayman & Mather (1955) and Mather & Jinks (1982).

An effort to join knowledge of the mechanisms involved in the inheritance of resistance to *O. nubilalis* was regarded as essential by breeders dealing with selection and breeding for resistant strains of maize. Thus in the earlier years Martson (1930) concluded from F3 data obtained from crosses between "Maize Amargo" and the variety "Michigan dent", that resistance to *O. nubilalis* was inherited simply. On

the other hand, Meyers *et al.* (1937) reported no evidence of a simple inheritance of resistance to maize borers. This was later supported by Patch *et al.* (1942), who reported that resistance to the borer was due to the cumulative effect of an undetermined number of multiple factors. Later Patch & Everly (1948) stated that the gene controlling plant reaction to *O. nubilalis* had a geometric rather than an arithmetic effect. Scholosberg & Beker (1948) working with sweet maize showed that incomplete dominance is probably due to the cumulative effects of several factors. Singh (1953) in his studies on the inheritance of maize borer reaction in certain resistant and susceptible inbred lines, concluded that the genetic effects for both leaf-feeding and overall damage was additive and these were in agreement with two factor pairs. However, Ibrahim (1954), using chromosomal interchanges, found that at least three genes were involved.

It was reported from studies on leaf feeding (Penny & Dicke, 1956) for the F3 and the backcross progenies of susceptible X resistant lines that three or more genes were controlling borer resistance with partial phenotypic dominance of susceptibility. The same conclusion was reached by Fleming *et al.* (1958).

Chiang & Hedson (1973) conducted studies on resistance to leaf feeding by *O. nubilalis.* They found that in eight inbreds used, the additive component appeared to be the most important in determining resistance. Klun *et al.* (1970) found that there was a highly negative correlation between the concentration of DIMBOA (2.4 dihydroxy-7-methoxy 2H-1.4benzoxazin-3(4H)-one) and resistance to leaf feeding by first-brood maize borer in dent maize. They also indicated that the

additive X additive epistatic effects, or both, were predominant in their diallel analysis for concentration of DIMBOA.

Later Scott *et al.* (1966) using reciprocal translocation techniques determined that the resistance in CI31A is dependent upon genes at loci on at least five chromosome arms. Different scientists found that both additive and nonadditive genes were important for insect resistance (Pathak, 1991; Pathak & Oithieno, 1990; Van Rensburg & Gevers, 1993; Ajala, 1992; Onukogu *et al.*, 1978).

It was stated by Pathak (1991) that increased levels of resistance were associated with significant yield reductions under artificial *O. nubilalis* infestation. Therefore, the selection criteria for resistance should include yield (Guthrie, 1989).

It is known that resistance to second generation *O. nubilalis* is controlled by at least five alleles (Onukogu *et al.*, 1978; Schon *et al.*, 1993).

The studies done during the past several decades on leaf feeding resistance in maize (first generation *O. nubilalis*), and to sheath-collar feeding by second generation *O. nubilalis*, indicate that various resistant inbreds may carry several factors conditioning resistance (Guthrie, 1987a). He came to the conclusion that leaf feeding by first generation *O. nubilalis* is conditioned by at least eight genes.

However, it is believed by most breeders that additive gene action, evaluating different crosses and using different sources of insect

resistance, indicate the use of recurrent selection as a viable procedure for the development of insect resistance (Barry *et al.*, 1983, 1984, 1985; Klenke *et al.*, 1986). The insect resistance is controlled by many genes located at different loci. The number of genes controlling the resistance rules out the possibility of using a backcross procedure to transfer resistance to susceptible maize genotypes (Sarges *et al.*, 1994; Guthrie, 1974; Guthrie, 1987b).

Tseng *et al.* (1984) used a recurrent selection breeding technique to reduce leaf-feeding damage by first-generation *O. nubilalis* and to increase DIMBOA content in a synthetic maize cultivar.

The adoption of modified backcross breeding methods to transfer the resistance into an agronomically desirable cultivar/inbred line was suggested since in both backcross generations a high proportion of resistant genotypes was realised by Pathak *et al.* (1989). They recommended that the manipulation of genes for resistance through conventional breeding methods should continue to be used to develop resistant hybrids and cultivars until genetic engineering techniques are perfected.

However, with the assistance of molecular probes to track movement of both favourable resistant alleles and recurrent parent alleles, the feasibility of backcross breeding for complexly inherited traits improves.

Using artificially infested field trials, molecular markers were identified that are associated with resistance to stalk damage by *O. nubilalis*. This process of developing a quantitative trait loci (QTL) model, was used at

Northrup King Company Research Centre (USA) to develop several QTL models for various sources of *O. nubilalis* resistance in a molecular marker-assisted breeding program (Sarges *et al.*, 1994).

Maize is an excellent species for QTL analyses. If QTLs can be identified in commercially used inbreds, the transfer of results into applied plant breeding programs should be facilitated. The success of hybrid maize breeding programs depends on efficient procedures to identify lines that produce superior hybrids. Nevertheless, evaluation of combining ability of new lines in extensive field tests is still the most costly and time-consuming part in modern hybrid breeding programs (Burr *et al.*, 1983).

The detection of significant association between genes conferring pest resistance and RFLP, AFLP and other markers, will be useful for a wide range of applications (Schon *et al.*, 1993). For breeders it is important to obtain a more profound understanding of the inheritance of polygenic pest resistance and its interrelation with other agronomically important traits in order to develop improved breeding strategies (Schon *et al.*, 1993).

2.6 - Marker-assisted selection

Although it has been demonstrated that resistance is conditioned predominantly by additive gene effects (Scott *et al.*, 1967; Jennings *et al.*, 1974), the exact number and location of resistance factors (loci) vary according to the source of resistance to be used. The possible value of using marker-assisted selection for improving insect resistance

must be estimated, as for each different resistance source utilised, molecular markers must be identified that are associated specifically with that source's insect resistance alleles. These markers need to be polymorphic so that they can differentiate between the alleles of the resistant and susceptible genotypes in chromosome regions linked to resistance genes (Sarges *et al.,* 1994).

Provided these conditions are met, molecular markers can be used to follow resistance alleles in the progeny of a cross between a resistant parent and the susceptible parent that is to be improved.

2.7- Advantages in using MAS

Molecular markers are being studied for their potential to enhance selection efficiency in plant breeding. They have been suggested as a means of direct selection for traits which have low heritability, are difficult or expensive to measure or require wide crossing for incorporation (Nienhuis *et al.,* 1987; Soller & Beckmann, 1983; Tanksley *et al.,* 1989). MAS has emerged as a strategy for increasing selection gains (Dudley, 1993; Lande & Thompson, 1990; Lande, 1992; Knapp, 1994a). With the help of molecular markers, introgression and pyramiding of resistance genes from exotic or agronomically acceptable germplasm may be generated with considerable savings in time (Schon *et al.,* 1993).

Techniques which are particularly promising in assisting selection for desirable characters involves the use of molecular markers such as random-amplified polymorphic DNAs (RAPD), restriction fragment

length polymorphisms (RFLPs); microsatellites; PCR-based DNA markers such as sequence characterised amplified regions (SCARs) ; amplified length polymorphisms (AFLPs); sequence tagged sites (STS) and inter-simple sequence repeat amplification (ISA), and amplicon length polymorphisms (ALPs), using F2 and backcross populations, near-isogenic lines, doubled haploids and recombinant inbred lines.

The essential requirements for marker-assisted selection in a plant breeding program are:

- markers should co-segregate or be closely linked (1cM or less in probably sufficient for MAS) with the desired trait;
- an efficient means of screening large populations for the molecular markers should be available. At present this means relatively easy analysis based on PCR technology.
- the screening techniques should have high reproducibility across laboratories, be economical to use and user-friendly.

Thus, recent developments in molecular marker technology together with the concept of marker-assisted selection are providing new solutions for selecting and maintaining desirable genotypes (Mohan & Suresh, 1997).

With MAS it is now possible for breeders to conduct many rounds of selection in a year. Molecular marker technology is now integrated into existing plant breeding programs all over the world in order to allow researchers to access, transfer and combine genes at a rate and a precision not previously possible (Mohan & Suresh, 1997).

In a breeding program the applicability of molecular markers depends on a fast detection method and on the specificity of the marker for the gene of interest in genetically diverse breeding material (Schachermayr *et al.,* 1997). In breeding for disease and pest resistance, at present the segregating populations derived from crosses between the resistant sources and otherwise desirable and productive genotypes are selected either at natural pest hot-spots, in artificially created pest nurseries or by infecting individual plants under controlled environments. Although these procedures have given excellent results, they are time consuming and expensive. Besides, there are always susceptible plants that escape attack. Furthermore, the pests have to be maintained either on the host or alternate hosts if they are obligate parasites. Screening of plants with several different pests and their biotypes simultaneously or even sequentially is difficult if not impossible. Availability of tightly linked genetic markers for resistance genes will help in identifying plants carrying these genes simultaneously without subjecting them to insect attack in early generations. The breeder would require a little amount of DNA from each of the individual plants to be tested without destroying the plant. Using the known set of primers for PCR, the products of the reaction would have to be run on agarose gels and the genotype of the individual plant for resistance or susceptibility could then be directly ascertained by the presence or absence of the marker band on the gel. Only the materials in advanced generations would be required to be tested in insect nurseries. Thus, with MAS, it is now possible for the breeder to conduct many rounds of selection in a year without depending on the natural occurrence of the pest and theoretically without the pest as well.

Insects are known to overcome resistance provided by single genes. Durability of resistance has been increased in several crops by incorporating genetic diversity of the major resistance genes. Cultivar diversification, cultivar mixtures, multilines and pyramiding of resistance genes have been successfully used. MAS for resistance genes (R) can be useful in all these approaches. Based on host-insect interaction alone it is often not possible to discriminate between the presence of additional R gene(s). With MAS new R gene segregation can be followed even in the presence of the existing R gene(s) and hence R genes from diverse sources can be incorporated in a single genotype for durable resistance (Yoshimura *et al.,* 1995).

2.8- Disadvantages of using MAS

Although the gains from marker-assisted index selection are theoretically greater than the gains from phenotypic selection (Lande & Thompson, 1990), quantitative trait loci (QTL) and MAS index parameter estimation errors, genetic drift, and disequilibrium between selected and unselected QTL can reduce the gains from MAS. This may lead to lower selection gains for MAS than for phenotypic selection, particularly in long range or recurrent selection experiments (Beavis, 1994, 1997; Bulmer, 1971; Dudley, 1993; Gimelfarb & Lande, 1994a,b, 1995; Knapp *et al.,* 1993; Knapp, 1994b; Lande & Thompson, 1990; Lande, 1992; Zhang & Smith, 1992, 1993).

The presence of different races or biotypes complicates the development and application of MAS. Markers developed for one pathotype or biotype may not have application to other locations in

which different pathotypes or biotypes occur, unless resistance is controlled by the same gene. Mohan & Suresh (1997) found that one of the major drawbacks is when the linked marker used for selection is at a distance away from the gene of interest, leading to cross-overs between the marker and the gene. This produces a high percentage of false-positives/ negatives in the screening process. The second problem is found associated particularly with procedures involving PCR with arbitrary primers which has relatively low reliability (5-10% error rate) (Weeden *et al.,* 1992).

Another of the major problems in using different marker technology is breeding expenses involved. Ragot & Hoisington (1993) compared the costs of three molecular marker protocols: chemiluminescent restriction fragment length polymorphism, radioactivity-based RFLP and RAPDs. Although their analysis focused on studies involving large numbers of probes/primers, and thus is not totally appropriate for MAS applications, their breakdown of costs for RAPD analysis indicated that nearly half of the costs could be attributed to DNA extraction and detection steps. Length DNA isolation protocols can be bypassed by using squashes of plant tissues as substrates for PCR (Langridge *et al.,* 1991).

2.9- Effectiveness in MAS

MAS should be most effective in the early generations of selection among progeny from crosses between inbred lines (Lande, 1992; Stromberg *et al.,* 1994). Heritabilities are usually lowest (because replications are limited and experimental unity tend to be small) and linkage disequilibrium is greatest in these generations (Falconer, 1981).

The paradox is that the power for mapping QTL decreases as heritability decreases and is lowest for traits where MAS has the greatest theoretical impact (Lande & Thompson, 1990; Lande, 1992). According to Wolfram (1989) the efficiency of MAS relative to phenotypic selection can be estimated by

$$Ec = Nps/Nmas = \log I0\,[1- Pr\,mas\,(1- \Phi[I])] / \log 10\,[1-Pr\,ps(1- \Phi[x])],$$

where Pr(mas) is the probability of selecting at least one progeny with a genotypic value (Gi) greater then g' among progeny with MAS index value (Ij) greater than I'. The factor $\Phi(i)$ is the area under a standard normal distribution below i, and Ec can be used to assess whether or not MAS is cost efficient for a specific breeding problem by comparing the cost per observation for phenotypic (Cps) and marker (Cmas) assays along with Nps and Nmas. For example if the cost per observation is 10 times greater for MAS than for phenotypic selection (Cmas/ Cps =10) and Ec =5, then phenotypic selection is twice as cost efficient as MAS ((Cmas/Cps)/(Nps/Nmas)=10/5 = 2), even though phenotypic selection requires five times as many progeny as MAS (Ec =5) to achieve the same breeding goal.

If QTLs exhibit significant epistatic interaction, marker-assisted selection should increase efficiency by facilitating the selection of genotypes with favourable alleles at both loci. Moreover, if screening for resistant genotypes is very costly and time consuming as in the case of *O. nubilalis* resistance, the combination of marker-assisted and phenotypic

selection should be superior to classical methods (Schon *et al.*, 1993; Lande & Thompson, 1990).

2.10- Heritability estimates for MAS

The accuracy of QTL and MAS index parameter estimates can be low when heritability is low and samples are small (Beavis, 1994, 1997; Gimelfarb & Lande, 1995). This problem is not unique to early generation MAS. Early generation phenotypic selection is seldom strongly advocated in crop plants despite the theoretical drawbacks of delaying selection (Geiger, 1984; Snape & Simpson, 1984; Sneep, 1977, 1984). Selection is frequently delayed to later generations because heritabilities and the statistical accuracy of progeny mean estimates tend to increase as the number of replications, generations, sites, and years of testing increase. Although organisms, traits, and circumstances differ greatly, there are two universal sampling problems: First, enough progeny must be tested and selected to ensure that at least one has a superior genotype (is fixed for more favourable alleles than the parents or has a genotypic mean exceeding a genotypic superiority threshold selected by the breeder). When the heritabilities of the selected traits are low or moderate and small samples of progeny are tested, the probability of selecting an outstanding genotype is very low (Robson *et al.*, 1967; Johnson, 1989). Secondly, selected progeny are mixtures of superior and inferior genotypes. The frequency of inferior genotypes in a selected sample of progeny increases as heritability decreases (Robson *et al.*, 1967). The usual strategy for sorting superior genotypes is "advanced testing".

If breeders had tools to increase heritability cost effectively, then breeding program outputs and efficiency could be greatly increased by testing fewer progeny per cross, culling inferior progeny early, and using higher selection intensities. The problem with implementing MAS, apart from QTL parameter estimation errors, is the cost difference between molecular marker and phenotypic assays for most traits. This difference should steadily decrease as the technology advances (Perlin *et al.,* 1995; Schwengel *et al.,* 1994; Vos *et al.,* 1996), and the advances in the technology should increase the merit of MAS as a strategy for increasing heritability.

Lande & Thompson (1990) described an optimum index for selecting individuals or lines (families) for a normally distributed quantitative trait. This index is a weighed sum of phenotypic and marker scores, with weights calculated as per an optimum selection index (Hazel, 1943). The vector of index scores for one trait is estimated by $I = bpX + bm$, where:

$$b = P^{-1}Gd = [\, bp, bm \,]$$

is a vector of index weights, x is an N x 1 vector of phenotypic scores, $m = \Sigma k \alpha k n k$ is an N x 1 vector of marker scores, N is the number of progeny tested, αk is the additive effect of the kth marker locus, nk is the number of favourable alleles at the kth locus ,

$$bp = \sigma^2 g - \sigma^2 m \,/\, \sigma^2 p - \sigma^2 m = 1 - p \,/ 1/h^2 - p$$

is the index coefficient for phenotypic scores,

$$bm = \sigma^2 p - \sigma^2 g \,/\, \sigma^2 p - \sigma^2 m = 1/h^2 - 1 \,/\, 1/h^2 - p$$

is the index coefficient for marker scores, $\sigma^2 g$ is the additive genetic variance between lines, $\sigma^2 p$ is the phenotypic variance between lines, $\sigma^2 m$ is the additive genetic variance associated with marker loci, $p = \sigma^2 m / \sigma^2 g$ is the proportion of the additive genetic variance associated with markers, and $h^2 = \sigma^2 g / \sigma^2 p$ is the heritability.

Therefore, the additive genetic effects of QTLs associated with linked molecular markers can be estimated by multiple regression of individual phenotypic value, z, on the number of copies of a particular allele (0,1 or 2) at the phenotypic marker loci.

Most of the important agronomic characters are controlled by several genes. The number of genes and their interactive effects controlling the expression of quantitative traits are poorly understood. Many QTLs have been identified by using DNA markers in different crop plants such as tomato (Paterson *et al.,* 1988; De Vicente *et al.,* 1993,) maize (Edwards *et al.,* 1992; Stuber *et al.,* 1992), and barley (Hayes *et al.,* 1993; Laurie *et al.*, 1995).

The MAS procedures are still being developed for many crop plants using different kinds of molecular markers. RFLPs were used by Smith *et al.* (1992), to compare the diversity among widely used hybrids in the USA. It was determined that the joint usage contribution for hybrids that seem to have a similar germplasm on the basis of their RFLP profiles. This work provided the insight that collective use of hybrids from two close genetic parents, can, therefore, reduce genetic diversity to an

extent equivalent to that contributed by disproportionate use of individual widely grown hybrids.

It was found that ALFP assays allow a more detailed assessment of cultivar relationships. The AFLP approach provides an important practical advance for DNA profiling and will play a major role in the effective management of germplasm resources. A specific future requirement is to enhance the understanding of genotypic-agronomic relationship through the identification of specific chromosome locations of oligo-and polygenic traits Smith *et al.* (1992).

Reamon-Bütner *et al.* (1997) have shown that the combined strategy using bulked segregant analysis and AFLP markers is an efficient method to identify tightly linked markers to the sex locus in asparagus.

Meksen *et al.* (1996) using such a combined strategy detected tightly linked AFLP markers to the *Phytophthora infestante* resistance gene R1 in tomato.

AFLPs have been used to identify intraspecific varieties in rice (Cho *et al.,* 1996), to determine the degree of relatedness between soybean accessions (Maughan *et al.,* 1996), assess genetic variation in endangered plants (Travis *et al.,* 1996), and distinguish morphologically identical *Bacillus anthracis* isolates (Keim *et al.,* 1997). In each case AFLPs revealed previously undetected levels of variability. Barret & Kidwel (1998) used AFLP to assess genetic diversity among wheat cultivars.

According to Zehr *et al.* (1992) molecular markers can be used to detect alleles in donor genetic material for improvement of existing cultivars or hybrids.

2.11- Mapping and characterization of QTLs

The concept that quantitative traits can be inherited as a result of the segregation of multiple genetic factors modified by environmental effects is seen as a resolution for the conflict between the Mendelian theory of particulate inheritance and the observation that most traits in nature exhibit continuous variation (Johannsen, 1909; Nilsson-Ehle, 1909; East, 1916).

Many traits in plants and animals are quantitative in nature, influenced by many genes. It has been, for a long time an important aim in genetics and breeding to identify those genes contributing significantly to the variation of traits within and between populations or species. Today, with advances of molecular technology, it is possible to use molecular markers to map QTL, (Xie C. *et al.,* 1999; Groh *et al.,* 1998; Zeng, 1994; Schon *et al.,* 1993; Davarsi *et al.,* 1993; Lander & Botstein, 1989).

The mapping of quantitative loci by means of molecular markers such as RFLPs and others, allows the detection, localization, and characterization of genetic factors contributing to the variation of polygenically inherited traits. So the development of molecular marker technology and the use of these markers in QTL analysis has become a powerful approach for studying the genetic and phenotypic basis of

complex traits (Edwards *et al.,* 1987; Paterson *et al.,* 1988; Williams & Neal, 1992). The key element from which the formal theory of QTL mapping is constructed is the conditional probability of a particular QTL genotype given an observed marker genotype: by crossing two inbred lines linkage disequilibrium is created between loci that differ between the lines, and this in turn creates association between marker loci and linked segregating QTLs.

2.12- Experimental designs for QTL analysis

Starting with two completely inbred parental lines, P1 and P2, a number of line-cross populations derived from F1 can not be used for QTL mapping. The F2 design examines marker-trait association in the progeny from a cross or (selfing) of F1s, while the backross design examines marker-trait association in the progeny formed by backcrossing the F1 to one of the parental lines. The F1 therefore can be used to create recombinant inbred lines (RILs) and double haploid lines (DHLs) which allow marker-trait association to be scored in a completely homozygous background and across multiple environments. The F2 design has an advantage over other designs using backcross, RIL, or DHL populations, because it generates three genotypes at each marker locus, which allows the estimation of the degree of dominance associated with detected QTLs.

Designs using the Ft population (formed by randomly mating F1s for t-1 generations) allow for even higher resolution of QTL map positions than do F2s, albeit at expense of decreased power of QTL detection. More complex designs can be considered when individuals are genotyped in

one population, while trait values are scored in a future population derived from the genotyped individuals.

Fisch *et al.* (1996) presented a general treatment for such designs, such as the F2:3 design where F2 individuals are genotyped and then selfed. The trait value associated with a genotyped individual is estimated by the mean value of the resulting F3 family. Marker trait association can be assessed using one two-or multiple-locus marker genotypes. Under a single marker analysis the distribution of trait values is examined separately for each marker locus. Each marker-trait association test is performed independently of information from all other markers, so that a chromosome with n markers is generally a good choice when the goal is simple detection of a QTL linked to a marker rather than estimation of its position and effects.

Under interval mapping (or flanking-marker analysis), a separate analysis is performed for each pair of adjacent marker loci. Interval mapping offer a further increase in power of detection and more precise estimates of QTL effects and position. Composite interval mapping (Zeng, 1993, 1994; Jansen, 1993b, 1994b; Jansen & Stam, 1994) considers a marker interval plus a few other well-chosen single markers in each analysis, so that n-1 tests for interval-trait associations are performed on a chromosome with n markers. Multipoint mapping considers all of the linked markers on a chromosome (Kearsey & Hyne, 1994; Hyne & Kearsey, 1995; Wu & Li, 1994, 1996).

2.13- Estimate models

The detection of QTL is actually based on several models of analysis that were developed to facilitate the difficult task of evaluation the results of marker-trait association estimates. The simplest known test for marker-trait association involves the comparison of the trait means of alternate marker genotypes. This is the basis for linear-model approaches for detecting QTLs mainly when only two genotypes are compared (such as with single marker backross, RIL or DHL- designs). This can be accomplished with a simple test (Sokal & Rohlf, 1995). Most designs however, involve more than two marker genotypes. In such cases all marker genotypic means (or some subset of them) can be compared by using standard linear-model approaches, such as ANOVA or regression.

While the linear models provided above use only marker means, ML (maximum likelihood) uses the full information from the marker-trait distribution and, as such, is expected to be more powerful. Despite the difficulties of using this method the better approach of using this model is to use specialised algorithms of which EM (expectation-maximisation) methods have been successful adapted to many of the mixture-model problems in QTL mapping, (Carbonell & Gerig, 1991; Luo & Kearsey, 1992; van Oijen 1992; Carbonell *et al.,* 1992; Luo & Williams, 1993; Weller, 1993; Jansen, 1992, 1993a, 1994a, 1996; Jansen & Stam, 1994; Churchil & Doerge, 1994).

Chapter 3

A study on the inheritance of resistance to *Busseola fusca* in maize

3.1- Abstract

The stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), is an important pest of maize in South Africa. Attempts have been made to improve the resistance of maize against this pest using available exotic sources of resistance. The main goal has been concerned with the transfer of resistance from the resistant sources to locally adapted germplasm. This is difficult to achieve because of the polygenic nature of the insect resistance. The objective of this study was to determine the inheritance of *B. fusca* resistance in maize and to compare the viability of known methods with other ways of transfer. A set of trials were conducted at the Grain Crops Institute (Potchefstroom) using artificial infestation of 18 crosses achieved from a combination of local inbred lines with two exotic sources of resistance (Antigua group) Mp706 and CML139. Although the dominance gene action was found not significant by the joint scale test, the line x tester analysis indicated high dominance variation, which indicates that dominant gene action together with additive gene action and non heritable interaction are important for *B. fusca* resistance in maize. Hybrids from crosses of local maize genotypes with exotic sources of resistance are useful in resistance breeding. A recurrent selection approach for stem borer resistance to *B. fusca* should be initiated in F3 generation, due to a

high variability noticed in earlier generations that complicates the phenotypic selection.

Key words: additive, *B. fusca*; dominance; gene effects; inheritance of resistance.

3.2- Introduction

The first sources of stem borer resistance were developed in Iowa, USA, deploying the antibiotic substance DIMBOA against the European corn borer *O. nubilalis* (Guthrie, 1973). Pioneering work done at Mississipi State University, using Antigua and southern US germplasm provided a number of inbred lines with multiple resistance to various stem borer species (Williams & Davis, 1984a; Williams & Davis 1984b; Williams *et al.,* 1990). Other significant developments occurred at Tifton, Georgia, where a number of inbred lines were developed with resistance to corn earworm, *H. zea* (Widstrom *et al.,* 1975; 1984; 1988a; 1988b). CIMMYT, Mexico developed various inbred lines with multiple resistance, also using Antigua group2 as source material.

A number of lines from each of these institutions were in time evaluated for resistance to *B. fusca* in South Africa. Based on assessment of larval survival and growth rate, pronounced antibiosis to *B. fusca* was found in Mississipi inbred lines Mp705, Mp706 and Mp707 (Van Rensburg & Malan, 1990; van Rensburg & Van den Berg, 1995; Van Rensburg, 1998). Studies also confirmed moderate levels of resistance in the inbred lines Mp706 and Mp707 reported by Fourie

(1984) which is of possible value as a source of resistance to *B. fusca*. The CIMMYT line CML139 was also found to be resistant to *B. fusca* in a series of trials for assessment of new sources of resistance to the stalk borers *B. fusca* and *C. partellus* (Van Rensburg & van den Berg, 1995). Various lines of Mississipi and CIMMYT origin have since been used to introgress resistance into elite South African germplasm.

Since it became known that genetic variability exists within the maize genome for borer resistance, knowledge about how the resistance is inherited became of importance. Once the sources of resistance were found it became necessary to investigate the genetics of the resistance in order to develop an efficient breeding program (Pathak *et al.*, 1989).

Up to date there has been limited success towards transferring genetic resistance into genetically desirable cultivars, partly due to inadequate knowledge of the genetic nature of resistance. The available information is either contradictory or inconclusive (Ortega *et al.*, 1980). Remarkable work was done in recent years, by American researchers studying *O.nubilalis* (Barry & Darrah, 1994; Hamilton *et al.*, 1994). They demonstrated how different mechanisms of resistance (preference, antibiosis, tolerance), contributes to the overall resistance underlining the importance of the sources of the genetic variability for the success of the insect resistance breeding program.

It was shown that both additive and non–additive gene effects are significant in the inheritance of insect resistance pertaining to all three parameters of stalk borer damage (Pathak & Othieno, 1990, 1991;

Van Rensburg, 1993, Van Rensburg & Van den Berg, 1995; Ajala 1992; Wiseman & Bondari, 1992). In the case of *B. fusca* a preliminary study indicated the resistance in the Mississipi inbreds to be additively inherited (Van Rensburg & Gevers 1993).

Although most recent research in this field of science has shown that the recurrent selection method should be a useful approach to accumulate the alleles for resistance, the polygenic nature of this trait complicates the task.

The objectives of this study were to:
Study the inheritance of resistance to *B. fusca* in maize through the phenotypic assessment of the gene expression in the F2 population after crosses of susceptible inbred lines with two different sources of resistance, underlining the polygenic feature of the resistance.

3.3- Materials and methods

Eighteen inbred lines from various genetic backgrounds were crossed to two resistant testers CML139 and Mp706.

The derivation of the lines used are provided in Table 2. The lines were chosen based on the following:
Previous evaluations for resistance to both *C. partellus* and *B. fusca* indicated the following levels of resistance: P28 (susceptible to both

Table 2 -Maize inbred lines and their derivations.

Genotype	Derivation
Mp706	MpS wCB-4 ; Mississipi
CML139	MP78:518 (Antigua Gp2 x RP Gp1), CIMMYT- Mexico
P28	Experimental, Potchefstroom (South Africa), cornbelt types
D5	Experimental, Potchefstroom (South Africa), D940y types
F2834T	Teko Yellow (South Africa)
P608	Experimental, Potchefstroom (South Africa)
I137TN	Natal yellow horsetooth x Teko yellow (South Africa)
K64R	Pride of Salina.
M162W	Experimental, $K64R^2$. B1138T, (South Africa).
M37w	$21A^2$ Jellicorse, South Africa.
P3	(M37W x 21A) (21A x T115) Potchefstroom (South Africa).
P4	K64R types.
Miacatlan	Experimental,
B73	Experimental, Cornbelt Iowa Stiff Stalk Synthetic
B37	Experimental, Cornbelt Iowa Stiff Stalk Synthetic
M017	Experimental, Cornbelt, Midland x T8ex Jarvis Golder Prolific
Oh43	Experimental, Cornbelt (Oh40B x W8) Lancaster Sure 2^{nd}
Va35	Cycle
179.1137TN	(C103xT8) T8=Lancanter Sure Crop x Jarvis Prolific
K0315y	Experimental, Potchefstroom.
	DO940y(-1-2)6.HtN

C. partellus and *B. fusca*), D5 (susceptible to *B. fusca*), 179-I137TN (moderately resistant to *B. fusca*), P608 (susceptible to *B. fusca*), I137TN (intermediately resistant to *B. fusca*), M37w, K0315Y (highly susceptible to both *B. fusca* and *C. partellus*), (Van Rensburg & Malan, 1990, Van Rensburg, 1993).

The other lines listed in Table 3 excluding CML139 and Mp706 have never been evaluated for resistance but could be regarded as susceptible, based on field observations.

The lines were grown in single rows in two parallel plots, in the field at Potchefstroom during 1998/1999 planting season. In each plot the lines were crossed with one of the two sources of resistance plot Lx1 (CML139) and plot Lx2 (Mp706), resulting in 36 F1 crosses.

During the winter of 1999 two rows of each F1 cross were grown at Nabana Research Centre (Mpumalanga province) and plants were self-pollinated to fix the gene effects.

The segregating F2 populations obtained from the self-pollinated crosses were planted at Potchefstroom during 1999/2000. A randomized block design with three replications was planted using a row width of 1.0m and the plants spaced 70 cm apart. At the six-leaf stage (three weeks after emergence) the plants were artificially infested with 20 first instar larvae of *B. fusca* using a bazooka dispenser (Wiseman *et al.,* 1980). The used larvae were obtained from

a laboratory reared colony, using the procedures described by Van Rensburg (1993).

The scale of 1 to 9 described by Davis & Williams (1986), was used to assess the crosses. The scores were given as follows:
1-no visible leaf injury or a small amount of pin or fine shot-hole type of injury on a few leaves, 2- small amounts of shot-hole type lesions on a few leaves, 3- shot-hole injury common on several leaves, 4- several leaves with shot-hole and elongated lesions, 5- several leaves with elongated lesions, 6- several leaves with elongated lesions about 2,5 cm long, 7- long lesions common on about one-half of the leaves, 8- long lesions common on about two thirds of the leaves and 9- most leaves with long lesions.
The scale can be divided into three categories: 1-3 resistant, 4-6 intermediately resistant and 7-9 susceptible.

The material was planted ear-to-row taking five ears per category in the 2000/ 2001 growing season. Large gene variability was expected in the first generations after crossing the resistant and susceptible inbred lines. Two hundred plants from each of 36 combinations were evaluated for three parameters of damage: leaf feeding damage, larval mass and internal plant damage. The two combinations P608 x CML139 and P608 x Mp706 were chosen to ascertain the different levels of damage by planting ear-to-row.

Plants were infested with 20 first instar larvae of *B. fusca* each and after 15 days they were evaluated for the three parameters of damage as indicated above, using a scale of 1-4 as described by Van

Rensburg (1993). Each of the 36 crosses was considered as an individual experiment and so separate analyses were performed for each cross. Agrobase Software was used for line x tester analysis to compare the variation among the 18 populations of each cross. Duncan's multiple range test was applied to compare all possible pairs of means. The chi-square test was performed to estimate the expected and observed generation means. The results were used to establish the gene effects in populations with four and five family means (P1, P2, F1, F2, and F3 in 34 crosses and P1, P2, F1, F2, F3; F4 in both P608 x CML139 and P608 x Mp706 crosses). The gene effects were evaluated following the procedure outlined by Hayman (1958) and Hayman & Mather (1955), using the joint scaling test for three and five parameter models to estimate m, [d]-additive gene effect, [h]-dominance gene effect and [i]-additive x additive type of gene interaction, [l]-dominance x dominance type of gene interaction. The additive x dominance type of gene interaction could not be determined in the absence of backcrosses, due to not having the necessary six families needed for the six parameter model of a trigenic epistasis fitting. The scaling test for the absence of epistasis was computed using the three comparisons of means (Powers, 1941; Hayman & Mather, 1955), with A= 2(P1 F1)-P1-F1, B= 2(P2 F1)-P2-F1, C= 4F2-2P1-P2-2F1, $\sigma^2p - \sigma^2g$.

The heritability for the three groups of data available was computed as follows:

In combinations with non epistasis:

(Broad sense) $h^2=[\sigma^2F2-(\sigma^2P\ 1+\sigma^2P2+\sigma^2F1)/3] \times 100/\sigma^2F2$,

$\sigma^2p = \sigma^2g + \sigma^2e$ and $h^2 = \sigma^2A/\sigma^2p$ (Narrow sense).

With: $\sigma^2e = (\sigma^2F1 + \sigma^2P1 + \sigma^2P2)/3$

and $\sigma^2g = \sigma^2F2 - (\sigma^2P1 + \sigma^2P2 + \sigma^2F1)/3$

Where: σ^2= variance, P1= mean of the parental inbred lines, P2= mean of the parental resistant lines, F1 and F2 are the mean of the first and second generations of the crosses between the inbred lines and the two sources of resistance, σ^2g= genetic variance σ^2p= phenotypic variance and σ^2e=environmental variance.

In the third group of data the heritability was computed as follows:

(Broad) $h^2 = VF2 - [(VP1)(VP2)]^{1/2} / VF2$

Where: VF2= Phenotypic variance among F2 plants

VP1 and VP2 = Phenotypic variance among plants of parents and the single-cross population.

(Narrow) $h^2 = 2[Cov\ PO/\sigma^2p] = \sigma^2A/\sigma^2p$

Where: $Cov\ PO/\sigma^2p$ = the regression of offspring on parent.

3.4- Results and discussion

Differences were found between the parental inbred lines for all parameters of resistance evaluated (Table 2). Results showed pronounced differences between the susceptible and the resistant parents for all parameters of resistance measured.

The parental resistant inbreds were almost free of larval and internal plant damage and showed low leaf feeding damage scores compared to the susceptible parental lines. The same differences were found in the F1 and F2 generations (Table 3).

Table 3-Generation means for measurement of resistance to *B. fusca* in parental lines of maize.

		Parameters of Damage			
Inbred lines		**Larval Damage**	**No of larvae**	**Larval Mass**	**Internal Damage**
1	P28	5.67 ac	2.24	144.99 ef	1.75 ef
2	I137TN	4.73 e	3.02 a	160.43 df	3.91
3	B73	5.88 ab	1.75 d	95.55 gh	2.89 bc
4	B37	5.50 acd	2.62 b	213.13 c	2.93 c
5	Mo17	4.37 efg	1.47	181.81 ce	3.07 ac
6	P608	4.23 efg	1.22	186.20 cd	2.33 d
7	F2834T	5.07 bce	1.95 c	248.31 a	3.23 a
8	D5Exp	4.47 defg	0.96 ef	183.62 c	2.92 c
9	Oh43	5.57 ac	1.05 e	126.95 fg	1.67 ef
10	Va35	3.48 gh	0.88 fg	130.18 f	1.23
11	Miacatlan	3.04 h	0.36 h	37.13 I	0.46 g
12	K64R	3.59 ghi	0.90 fg	162.30 df	1.93 e
13	P3	6.43 a	3.00 a	250.56 a	3.17 ab
14	P4	5.59 ac	2.53 b	224.19 ab	3.50
15	M37W	5.15 bce	2.03 c	135.49 f	2.85 c
16	M162W	5.04 bcf	0.83 g	78.74 h	1.69 f
17	KO315Y	5.87 ab	1.67 d	213.46 c	2.50 d
18	179 I137TN	5.96 ab	3.35	151.39 ef	2.37 d
19	CML139	3.97 fh	0.29 h	29.55	0.75
20	Mp706	2.74 I	0.74	41.17 I	0.40 g

Means whithin columns followed by the same letters do not differ significantly at P=0.05 according to Duncan's multiple range test.

The results also showed promising heterosis. The F1 hybrids were almost free of larvae and showed very low internal damage. A high degree of resistance to *B. fusca* was observed in both crosses with slightly better performance in crosses with CML139.

In studies on resistance to *O. nubilalis* in maize it was found that leaf feeding by first generation borers was dominant (Ibrahim, 1954) and for internal damage (stem tunneling) it was dominant in the second generation (Jennings *et al.*, 1974). In hybrids, the heterosis is determined by the frequency of resistance genes and the degree of genetic diversity between parents involved in the crosses. In this case it seems that the significant heterosis for resistance in crosses between the local susceptible inbred lines and the exotic resistant CML139 and Mp706 may be due to the genetic diversity of both parents for resistance.

The good performance of the hybrids was totally weakened in the F2 generation (Table 3), where results showed very poor performance of the crosses. In some cases the crosses were less resistant than the parents. Using a scoring system (in categories 1-9) for leaf feeding damage in the F3 and F4 provided a very different picture (Table 4). The F2 generation heritable variance was possibly added with non-heritable variance that masked the real phenotypic expression of the resistance. The continued variation in the F3 generation (with less variability) and more clear differentiation from the parents is in accordance with Mather's (1958) postulate about increased variability in the F2 generation for inheritance of polygenic traits. The results

Table 4- Genetration means for measurement of resistance to *B. fusca* in line x tester crosses of maize.

Inbred lines		LEAF FEEDING DAMAGE				Parents
		x CML 139 (RP1)		x Mp706 (RP2)		
		F1	F2	F1	F2	
1	P28	4.16	7.67	2.89 d	5.85bce	5.67 ac
2	I137TN	2.43 bcd	5.12 g	3.27 cd	5.43 e	4.73 e
3	B73	2.57 bc	6.41 a	2.63 ef	5.51 e	5.88 ab
4	B37	2.43 bcd	5.63 cef	3.33 c	5.50 e	5.50 acd
5	Mo17	2.11 cd	6.08 ac	3.48 b	5.36 e	4.37 efg
6	P608	2.20 bcd	6.01 ac	3.10 d	6.29 ab	4.23 efg
7	F2834T	2.00 cd	5.28 eg	3.03 d	5.83 bc	5.07 bce
8	D5Exp	2.35 bcd	6.09 ac	2.85 d	5.66 c	4.47 defg
9	Oh43	3.58 a	6.06 ac	2.76 de	5.40 e	5.57 ac
10	Va35	2.70 b	5.48 deg	3.03 d	5.65 c	3.48 gh
11	Miacatlan	2.52 bc	5.43 eg	3.13 d	5.61 de	3.04 h
12	K64R	2.22 bcd	5.39 eg	2.13 f	6.04 acd	3.59 ghi
13	P3	3.41 a	6.27 ab	2.87 d	5.32 e	6.43 a
14	P4	2.52 bc	5.74 ce	4.03 a	6.26 ab	5.59 ac
15	M37W	2.21 bcd	6.99	3.47 b	6.51 a	5.15 bce
16	M162W	2.33 bcd	5.94 bcd	4.20 a	6.15 ac	5.04 bcf
17	KO315Y	2.05 cd	5.19 fg	2.81 d	4.99	5.87 ab
18	179 I137TN	1.92 d	5.28 d	3.40 bc	4.94	5.96 ab
19	CML139					3.97 fh
20	Mp706					2.74 I

RP1- (resistant parent 1), RP2- (resistant parent 2) Means within columns followed by the same letters do not differ significantly at P=0.05 according to Duncan's multiple range test.

suggest that selection for *B. fusca* resistance should not be initiated in the F2 generation but from the F3 and onwards, when the segregating variability is supposed to be lower. The same perception arises from the expected and observed generation means obtained by the Chi-square test (Table 4). The computed Chi-square test values were in part highly significant, indicating the presence of non-allelic interaction (Hayman, 1958). In the case of non- significant Chi-square values it was possible to fit the additive x dominant model of gene action. The additive gene action was found significant in most of the cases but the dominance was found to be non-significant in all cross combinations.

Nevertheless, the values showed that both additive and dominance gene action are of importance to *B. fusca* resistance in maize (Table 5). Having all six generations (P1, P2, F1, F2, F3 and F4) in combinations of P608 with the two exotic sources of resistance CML139 and Mp706, made it possible to estimate the epistatic interaction by means of a proposed five parameter model (Hayman, 1958; Hayman & Mather, 1955; Mather, 1955). The results indicated not only additive and dominance gene action but also the importance of non-allelic interaction. The use of the five parameter model derived additive x additive and dominance x dominance gene interactions (Table 6) the achieved d' (additive gene action) still associated with j- (additive x dominance) non-allelic interaction. The absence of backrosses made it impossible to separate these two parameters.

For combinations with non epistasis ($X^2 < 11.34$) df=3, the dominance is expressed by h= 2F1 - 2F2, d^=d, m^=m. The fact that [l]= P1 + P2

Table 5- Chi-square (df=3) for comparison of predicted and observed generation means and its significance in crosses of susceptible inbreds to two sources of resistance

Inbred lines	X CML139		X Mp706	
F2 Population	Mean	Chi-Square	Mean	Chi-Square
P28	7.68	292.28**	5.85	39.16**
I137TN	5.12	12.59**	5.43	9.63
B73	6.41	66.88**	5.51	6.43
B37	5.63	21.48**	5.50	22.27**
Mo17	6.08	61.52**	5.36	10.91
F2834T	5.28	7.71	5.83	45.28**
D5Exp	6.09	52.48**	5.66	24.00**
Oh43	6.05	58.32**	5.40	14.32**
Va35	5.47	9.39	5.65	23.71**
Miacatlan	5.43	15.39**	5.61	34.34**
K64R	5.39	15.47**	6.04	59.07**
P3	6.27	64.80**	5.31	4.63
P4	5.74	29.71**	6.26	82.48**
M37W	6.99	173.07**	6.51	108.00**
M162W	5.94	41.71**	6.15	70.83**
KO315Y	5.19	4.99	4.98	2.08
179 I137TN	5.27	26.19**	4.94	2.04
P608	5.47	9.39	5.65	23.71**

**-P= 0.05, *-P=0.01 levels of significance by t test.

Table 6- Estimates of mean genetic components for mean (m), additive (d), and dominant (h), of leaf feeding damage caused by *B. fusca* first instar larvae feeding on crosses of local susceptible inbred lines of maize with two sources of resistance, CML139 and Mp706.

	X CML139 (RP1)						X Mp706 (RP2)					
Progeny	m	SE	d	SE	h	SE	m	SE	d	SE	h	SE
I137TN							5.27	0.16	9.59*	0.48	-4.32	5.61
B73							5.35	0.16	11.1**	0.21	-5.76	7.64
Mo17							5.22	0.14	8.98	0.73	-3.76	6.96
F2834T	5.13	0.17	11.71*	0.72	-6.58	6.79						
Va35	5.31	0.16	10.87**	0.13	-5.56	7.68						
P3							5.15	0.17	10.1**	0.13	-4.9	7.69
KO315Y	5.14	0.13	11.42*	0.57	-6.28	6.74	4.78	0.16	9.14**	0.21	-4.36	7.30
179 137TN							4.78	0.16	7.86	0.88	-3.08	7.84

RP1= resistant parent 1, RP2=resistant parent 2.

SE= standard error.

* P=0.05 and ** P=0.01 of significance by t- test.

Table 7- Estimates of mean genetic components (m), additive (d), dominance (h), and non-allelic interaction (i-additive x additive, l-dominance x dominance) parameters for leaf feeding damage caused by *B. fusca* first instar larvae feeding on crosses of susceptible inbred lines with two resistant lines CML139 and Mp706 respectively.

P608		x CML 139 (RP1)		P608		x Mp706 (RP2)	
Generations	Mean			Generations	Mean		
P1	2.74			P1	3.97		
2	4.23			P2	4.23		
F1	2.20			F1	3.10		
F2	6.01			F2	6.29		
F3	3.01			F3	2.68		
F4	3.04			F4	2.68		
		m	5.94			m	6.14
		d'	-1.22*			d'	-1.52*
		h	-1.92			h	-1.00
		i	6.93**			i	11.66**
		l	-12.63**			l	-10.76**
		Broad	Narrow			Broad	Narrow
		h^2=0.64	h^2 =0.08			h^2 = 0.54	h^2 =0.03

P1=susceptible inbred line P608, P2- mean of resistant parents (CML139 and MP706).
*P=0.05 and **P=0.01 significance by t- test.

Table 8-Scaling test for the absence of epistasis in combinations with X^2 <11.34.

	x CML 139 (RP1)						x Mp706 (RP2)					
Progeny	A *	SE	B*	SE	C*	SE	A *	SE	B*	SE	C*	SE
I137TN							22.9	11.2	18.7	9.4	1.75	6.7
B73							25.4	12.7	14.3	7.6	-0.35	6.3
Mo17							22.6	11.0	20.2	10.0	1.77	6.6
F2834T	14.2	4.5	0.74	6.5	3.62	6.7						
Va35	17.2	8.8	9.36	5.4	4.78	6.6						
P3							28.8	13.9	16.0	8.3	-1.77	7.1
KO315Y	17.7	9.7	0.83	2.2	1.18	6.8	26.6	13.0	15.5	8.1	-1.63	6.7
179 I137TN							38.5	17.7	19.6	9.7	-5.47	7.0

SE= standard error. RP1= resistant parent 1, RP2= resistant parent 2.

* - A, B and C were not significant at P=0.05 by t- test.

Table 9-Estimates of heritability in the broad and narrow sense for the F2 generation of crosses of susceptible inbred lines and two sources of *B.fusca* resistance.

	CML139 X			X Mp706		
Progeny	h^2			h^2		
	Broad %	Narrow %	Se	Broad %	Narrow %	Se
P28	16.7	11.0	0.14	53.6	17.0	0.18
I137TN	81.0	42.0	0.19	65.1	3.0	0.17
B73	17.4	9.0	0.15	44.0	2.0	0.16
B37	60.9	35.0	0.14	43.9	3.1	0.13
Mo17	54.4	10.0	0.15	19.9	4.0	0.14
P608	69.5	8.0	0.16	55.4	3.0	0.15
F2834T	56.8	7.0	0.17	61.9	3.0	0.19
D5Exp.	76.2	7.0	0.17	71.9	13.0	0.17
Oh43	7.0	10.0	0.14	39.6	19.0	0.17
Va35	43.8	8.0	0.16	19.0	3.0	0.16
Miacatlan	41.9	8.0	0.17	50.7	3.0	0.19
K64R	28.0	8.0	0.16	22.4	15.0	0.16
P3	21.5	32.0	0.14	38.6	8.0	0.15
P4	46.0	13.0	0.14	39.3	20.0	0.16
M37W	21.8	13.0	0.13	28.7	3.0	0.15
M162W	53.0	9.0	0.15	33.6	23.0	0.16
K0315Y	57.6	4.2	0.13	60.3	2.0	0.16
179I137TN	36.7	13.0	0.12	51.4	10.0	0.16

Table 10- General combining ability (GCA) and specific combining ability (SCA) effects from line x tester analysis of eighteen inbred lines and two testers for *B. fusca* resistance in maize.

Progeny	X CML139		X Mp706	
	SCA	GCA	SCA	GCA
P28	0.81	0.98	-0.81	0.98
I137TN	-0.32	-0.45	0.32	-0.45
B73	0.37	0.19	-0.37	0.19
B37	-0.24	-0.20	0.24	-0.20
Mo17	0.26	-0.06	-0.26	-0.06
P608	-0.25	0.36	0.25	0.36
F2834T	-0.38	-0.23	0.38	-0.23
D5Exp.	0.13	0.10	-0.13	0.10
Oh43	0.22	-0.06	-0.22	-0.06
Va35	-0.20	-0.23	0.20	-0.23
Miacatlan	-0.20	-0.27	0.20	-0.27
K64R	-0.43	-0.07	0.43	-0.07
P3	0.45	-0.07	-0.45	-0.07
P4	-0.41	0.17	0.41	0.17
M37W	0.14	0.98	-0.14	0.98
M162W	-0.21	0.25	0.21	0.25
K0315Y	-0.01	-0.71	0.01	-0.71
179I137TN	0.07	-0.68	-0.07	-0.68

+ 2F1 - 4F2 (means) or [i]= P1 + P2 + 2F2 - 4F3 was significant (Table 6) indicates non-allelic interaction. Wiseman & Bondari (1992) quoted this interaction as a possible indication that stem borer resistance is controlled by several pairs of genes at different loci. Likewise it was found that [d']=P1 -m -1/2 h + i + 1/2 l was significant and [h]= 2F1 - 2F2 - 1/2 l not significant in both combinations of crosses. The scaling test for the absence of epistasis was performed to clarify the above controversial situation. The estimates A=2(P1 F1)–P1-F1; B=2(P2 F1)–P2- F1 and C= 4F2–2P1-P2-F1 was found to differ from zero but not significantly at P=0.05 (Table 7). This indicates negligible one locus non-allelic interaction but does not exclude non-allelic interaction for two or more loci (Kempthorne, 1957).

The heritability was estimated in three different ways because of the three different kinds of available data. This was in agreement with the results of the line test analysis that presented high dominance genetic variance. In combinations with non-epistasis in the broad sense, heritability was computed as the difference of the variances of P1, P2, F1 and F2:

$h^2= \sigma^2F2 - (\sigma^2P1+\sigma^2P2+\sigma^2F1)/3*100/\sigma^2F2$, and in the narrow sense $h^2=\sigma^2A/ \sigma^2P$, with $\sigma^2P= \sigma^2e + \sigma^2d + Vs$ and $s\sigma^2$=Cov (H.S), $\sigma^2d= \sigma^2e$-Cov (F.S).

The second group of data showed very high epistasis interaction (X^2 >11.34). Not having the necessary six families to derive the non allelic interaction, the heritability in the broad and narrow sense was computed in the same way as for the previous group. Similar to the

first group of data the heritability in the broad sense too, was very high for both crosses indicating high genetic variance (Wiseman & Bondari, 1992) while the estimated narrow sense heritability was very low, indicating non-additivity of the gene effects. The heritability in the broad and narrow sense and standard errors are shown in Table 8.

The third group of data comprised six populations namely, P1, P2, F1, F2, F3 and F4 respectively. This allowed for the derivation of the gene interaction based on the significant result of the X^2 test with df=3. This was three times less than the number of the families used in the joint-scaling test (five parameter model) (Hayman, 1958). The heritability in the broad sense was also found to be high in this group. It was computed as: $\sigma^2F2 - [(\sigma^2P1)(\sigma^2P2)]½ / \sigma^2F2$ (Mahmud & Kranur, 1951). The heritability in the narrow sense was computed to estimate the fraction of the genetic inheritance due solely to additive genetic variance for one locus: It was computed as, $h^2= 2[Cov PO/\sigma^2p]$. The result achieved with the narrow sense heritability for all combinations was very low (Table 9) this indicated that there was not additivity of the gene effects for resistance at one locus in most of the maize crosses. Possible exceptions were combinations of Mp706 with I137TN, B37, P3 and KO315Y.

Results of the SCA estimated by line x tester analyses show that in most cases it was greater than GCA, indicating good heterosis (Table 9). Although this indicator was found high for most of the combinations, the significant difference (Table 4) between the two combinations (X CML139 and X Mp706) was an important indicator

that the inheritance depends on the source of resistance and the inbred line to be used in the combination. This suggests that each inbred line possesses one or more of the possible loci of resistant genes assumed to compound the polygenic pool of loci responsible for the insect resistance. The percentage of selectable resistant plants from the whole population was computed as an indicator of the real inheritance of resistance, to be selected from the three populations P, F1 and F2 in the two different combinations (Table 11). The result indicated that in the F2 populations the ratio of selectable plants was in most of the cases less than those selected from the parental lines. This confirms the assumption that selection for resistance should preferable be initiated in the F3 and later generations.

Yield is a good indicator of the economic value of *B. fusca* resistance in maize. In this study the grain yield from infested and non-infested plots were determined for the F1 population (resistant) and the parental lines (18 susceptible and two resistant). The result indicated slight yield loss differences between infested and non-infested plants in the F1 population of both combinations. Otherwise, pronounced differences were found between infested and non-infested plots of all parental lines (Table 3).

A better understanding of the inheritance of *B. fusca* resistance would greatly increase the possibility of successes for breeders in a breeding program for variety improvement.

Table 11- Percentage selectable resistant plants from the F2 population in crosses of eighteen inbred lines with the two sources of resistance CML139 and Mp706.

Inbred lines		LEAF FEEDING DAMAGE				Parents %
		x CML 139 (RP1)		X Mp706 (RP2)		
		F1 (%)	F2 (%)	F1 (%)	F2 (%)	
1	P28	32.0	2.0	67.9	21.0	19.1
2	I137TN	89.3	32.0	72.4	22.0	13.3
3	B73	88.5	8.0	85.2	19.5	20.0
4	B37	91.3	14.5	66.7	14.0	3.7
5	Mo17	96.4	12.5	59.1	16.5	40.7
6	P608	96.6	14.0	63.3	8.5	25.9
7	F2834T	86.2	22.0	70.0	23.0	28.6
8	D5Exp	100	15.0	81.5	20.0	36.7
9	OH43	61.5	11.5	80.9	22.3	32.1
10	Va35	85.2	19.5	80.0	15.5	46.7
11	Miacatlan	96.3	20.5	63.3	25.5	55.6
12	K64R	73.9	22.0	90.0	16.0	51.9
13	P3	70.4	10.0	70.0	24.0	20.0
14	P4	81.5	14.0	40.0	13.0	16.7
15	M37W	92.9	2.5	60.0	10.0	11.1
16	M162W	100	13.5	46.7	13.0	11.1
17	KO315Y	100	19.5	75.0	26.0	3.7
18	179 I137TN	100	11.5	56.7	23.0	3.3
19	CML139					81.5
20	Mp706					36.7

RP1=resistent parent 1, RP2=resistant parent2.

Table 12- Yield losses (grams) caused by *B. fusca* larvae feeding on 18 susceptible inbred lines and two resistant sources of resistance CML139 and Mp706.

Inbred lines	Losses (mg) %/plant		
	Parent lines	xCML139 F1	xMp706 F1
P28	33	25	37
I137TN	50	10	38
B73	56	20	16
B37	44	0	6
Mo17	40	0	0
P608	46	0	0
F2834T	33	27	25
D5Exp	60	5	42
Oh43	50	0	24
Va35	25	0	20
Miacatlan	33	0	0
K64R	41	0	8
P3	73	0	18
P4	67	48	17
M37W	67	7	0
M162W	78	24	23
KO315Y	50	17	37
179 137TN	63	10	37
CML139	16		
Mp706	19		

3.5- Conclusions

The result that *B. fusca* resistance in maize is inherited differently by inbred lines related to the source of resistance, indicates that more information about the inheritance of insect resistance can be achieved, if line x tester assessment includes more sources of resistance. It could also be seen that more local and more adapted sources of resistance should be used to avoid the false high heterosis caused by the genetic diversity in the F1 population. The non-linkage perception indicated by low heritability in the narrow sense, prescribes that to achieve better understanding on how resistance is inherited, trials with a balanced design to avoid the environmental interaction should be conducted. It is concluded that the gene effects for insect resistance in maize is not fixable with ease due to the presence of significant non-heritable gene interaction present in the first generation after crossing. In order to find improved methods to fix the genes of interest further studies on the perfection of markers are needed.

In a breeding programme, the improvement of a given susceptible line for resistance could be achieved in a shorter period of time by crossing the line to more than one resistant source, and recombining between crosses after selection in the F3 generation.

Chapter 4

Genetic expression of antibiosis to *Busseola fusca* and its possible correlation with damage features in maize.

4.1- Abstract

An understanding of how the three parameters of insect resistance in maize against *B. fusca* are inherited in certain proportions could help breeders design more balanced breeding programs. The present study was conducted to estimate the possible relationship between the three parameters of resistance assessment in maize, leaf feeding damage, internal plant damage and larval mass gain. The research was conducted at the Grain Crops Institute (Potchefstroom) through the assessment of mass gain by first instar larvae feeding on 18 susceptible inbred lines and their crosses with two resistant sources Mp706 and CML139 respectively. High variability was found in the F1 generation for leaf feeding. Simple correlation and multiple regression coefficients were calculated for generations F1, F3 and F4. There was an important correlation between the three parameters of *B. fusca* resistance assessment. A negative correlation between larval mass and the leaf feeding in the F1 generation (resistant) and positive correlation for the same parameters in parental lines (susceptible) was an indication of the presence of an inhibitor factor in resistant inbred lines.

Key words: *Busseola fusca*, resistance, maize, antibiosis, inheritance.

4.2- Introduction

The maize stalkborer is generally considered the most destructive of all insects attacking maize in South Africa (Smithers, 1960; Rose, 1962; Walters, 1975). In breeding maize resistant to *B. fusca* questions arose as to the efficacy of different methods of screening and the determination of the mechanisms of resistance.

The first report on the existence of antibiosis to stalk borers by Walter (1957) led to studies on mechanisms of insect resistance in maize. Different studies were conducted to estimate antibiosis responses (Straub & Fairchild, 1970; Wiseman & Isenhour, 1990; Wiseman & Isenhour, 1991; Wiseman *et al.*, 1992a). All these authors found valuable information about antibiosis through larval mass assessment.

Phenotypic plant responses to insect feeding have been determined in a number of ways. Most programs using recurrent selection are based on leaf feeding damage, estimation on a scale of 1-9 (Guthrie *et al.*, 1960; Davis & Williams, 1986; Davis, 1987), characterizing inbred material or hybrids usually involves number of damaged internodes, measuring length of stem tunneling, plant stunting, larval survival and larval mass (Williams *et al*, 1978; Scott & Davis 1978; van Rensburg, 1982; Barrow, 1985; van Rensburg & Malan, 1990; van Rensburg & Gevers, 1993).

A number of researchers have employed nutritional indices to study the intake, digestibility and efficiency of conversion of food sources by different lepidopterous species (Waldbauer 1968; Chou et al., 1973; Dahlman, 1977; Davis *et al.*, 1989; Kumar, 1993; Ng *et al.*, 1993). The results could be used to determine whether plant resistance affects insect behaviour or metabolism.

Valuable documentation on antibiosis is available from research on the biological effects of antibiosis resistance to corn earworm *H. zea* (Starks & Mc Millian 1967; Wann & Hills 1966; Wiseman *et al.,* 1976, 1978, 1983; Wilson *et al.*, 1984). The measured effects included mortality of larvae (Widstrom *et al.*, 1979), reduced mass of larvae 8-10 days after egg hatching (Wiseman *et al.,* 1983) and reduction in populations (Wiseman *et al.*, 1978). Later, Wiseman & Isenhour (1990) showed additional biological effects of antibiotic maize silks on *H. zea,* including increased duration of pupation, decreased mass of pupae, and decreased fecundity over four generations. An increased duration of the larval stages as well as a smaller head capsule in association with feeding on meridic diets containing antibiotic silks, was shown by Wiseman & Isenhour (1991). Although it has been suggested that a portion of antibiotic factor in Zapalote Chico was "maysin," a luteolin-c-slycoside (Waiss *et al.*, 1979), the real genetic and chemical structure is still not understood.

Recently it was found that the 33Kda cysteine in callus initiated from maize embryos was correlated with inhibition of fall armyworm, *Spodoptera frugiperda* larval growth (Jiang *et al.,* 1995). Differences in cysteine proteinase isolated from callus of the resistant Mp708 and

susceptible line Ab24E was ilustrated. The difference in performance of the two enzymes that code for the two proteins, led to the assumption that the presence of a 33-Kda protein with a relatively high cysteine proteinase activity may be required to inhibit larval growth in callus. Ingestion of cysteine proteinase could directly harm the insect digestive system by destroying gut proteins. Alternatively, the proteinase could catalyze a reaction leading to a substance that is toxic to larvae. The finding that the 33-KDa cysteine proteinase is correlated with the inhibition of larval growth on callus (Jiang *et al.,* 1995), is a great achievement if it can be proved that this enzyme is a direct retardent of larval growth of the insect. These findings led to the need for more information about the catalytic process encoding the protein. It was found that 33-KDa cystein proteinase encoded by mir1 is expressed intensively in callus and comprises 1% of the total protein (Pechan *et al.,* 1999). When *S. frugiperda* larvae are reared on callus from resistant genotypes expressing the 33-KDa cysteine proteinase they weigh 50% less than those reared on callus of susceptible genotypes (Jiang *et al.,* 1995).

It was demonstrated too, that a low level of 33-KDa cysteine proteinase is present in whorls of resistant genotypes and that it dramatically increases in abundance after wounding or insect feeding (Pechan *et al.,* 1999).

The above are examples of how knowledge on the mechanisms of reistance in a given resistant source may serve to facilitate breeding programmes. The present study concerns plant responses to insect attack, with the intention to evaluate the possible correlation of

antibiosis with the phenotypic expression of resistance in the F1, F3 and F4 generations, after crossing the two resistant sources Mp706 and CML139 to 18 susceptible inbred lines.

4.3- Materials and methods

Progenies (F1, F3 and F4) from crosses between two sources of resistance (Mp706 and CML139) to 18 susceptible inbred lines were used in the study. Mp706 is resistant to *B. fusca.* It was derived from the Antigua group 2 population by Williams & Davis (1984a) as a source of resistance to Southwestern corn borer, *Diatrea grandiosella* and the Fall armyworm, *Spodoptera frugiperda*. CML139 is a new source of resistance to *B. fusca* identified from an assessment of new sources of resistance developed by CIMMYT, Mexico (Van Rensburg & Van den Berg, 1995).

The 18 susceptible inbred lines are representative of local and cornbelt maize genotypes prominent in most maize programes. These were classified as susceptible, moderately resistant or highly susceptible as shown bellow:

1- P28 (susceptible to both *C. partellus* and *B. fusca*)
2- D5 (susceptible to *B. fusca)*
3- F2834T (susceptible to *B. fusca*)
4- P608 (susceptible to *B. fusca*)
5- I137TN (intermediately resistant to *B. fusca*)
6- K64R (susceptible to *B. fusca*)
7- M162 (susceptible to *B. fusca*)

8- M37W (highly susceptible to *B. fusca*)

9- K0315Y (highly susceptible to *B. fusca*)

10- P3 (susceptible to *B. fusca*)

11- P4 (susceptible to *B. fusca*)

12- Miacatlan (susceptible to *B. fusca*)

13- B73 (susceptible to *B. fusca*)

14- B37 (susceptible to *B. fusca*)

15- Mo17 (susceptible to *B. fusca*)

16- Oh43 (susceptible to *B. fusca*)

17- Va35 (susceptible to *B. fusca*)

18- 179 137TN (susceptible to *B. fusca*)

A field trial, with three replications, including F1, F2 and F3 populations were planted during 1999/2000 at Potchefstroom. At the six-leaf stage the plants were artifitialy infested with 10 first instar larvae of *B. fusca* each using a bazooka dispencer (Wiseman *et al.*, 1980). Larvae were obtained from a laboratory reared colony (van Rensburg, 1993). Leaf feeding damage was assessed two weeks after infestation, using a scale of 1-9 as discribed by Guthrie *et al.*(1960). Three weeks after infestation, plants were dissected longitudinally. Larvae were weighed to determine the mass gained after three weeks of feeding. Internal plant damage was assessed by counting the number of damaged internodes. Generation means and standard errors were computed for all generations involved in the trial. Simple correlation coefficients were calculated between the three parameters of insect damage (leaf feeding, number of larvae and larval mass).

Table 18- Generation means for three parameters of *B. fusca* damage in maize hybrid populations derived from two cross combinations between 18 susceptible inbred lines and each of CML139 and Mp706.

Progeny F1	PARAMETERS OF DAMAGE					
	x CML 139 (RP1)			x Mp706 (RP2)		
	NL	LW	ID	NL	LW	ID
P28	2.48 ab	98.00 ac	1.33 bcde	2.03 ac	50.95 efg	0.37 f
I137TN	0.97 fg	54.39 defg	1.60 ad	3.69	79.55 bcd	1.87 a
B73	1.97 bc	98.03 ab	1.25 bcdf	2.66 a	58.78 dg	0.53 efg
B37	1.92 bc	60.91 bc	1.00 cdg	2.22 ab	106.15 ab	1.56 ab
Mo17	1.86 bd	62.22 bc	0.62 efg	1.17 ce	96.16 ac	0.80 cdef
P608	2.85 a	206.34	2.06 a	1.17 de	90.66 ad	1.23 bc
F2834T	0.69 h	35.66 fg	0.62 fg	2.22 ab	80.69 adf	1.06 bf
D5Exp	1.71cde	84.94 acd	1.50 ac	1.99 ac	84.44 ade	1.13 bd
Oh43	0.69 h	28.53 g	0.73 efg	0.78 ef	52.56 efg	0.33 f
Va35	0.67 h	46.75 defg	0.50 g	1.46bce	93.72 ad	1.00 bf
Miacatlan	0.76 g	11.65	0.73 fg	1.63bcd	59.77 cdg	0.47 f
K64R	1.57 cdf	69.29 bcf	1.00 cdg	1.06 de	30.43 g	0.13 g
P3	1.04 efg	96.29 ac	1.73 ab	0.10 f	0.10	0.00 g
P4	1.39 cdf	80.85 ace	1.00 cdg	0.63 e	110.96 a	1.06 bf
M37W	1.31dgh	106.63 a	0.92 cdg	1.96 ac	71.98 bcd	1.07 be
M162W	2.52 ab	65.64 bcg	1.14 bdg	4.56	224.60	2.88
KO315Y	0.68 h	23.88 g	0.33 g	0.67 e	36.31 fg	0.12 g
179 137TN	0.41 h	44.87 efg	0.66 cfg	0.10 f	0.10	0.18 g

RP1=resistant parent 1, RP2= resistant parent 2, NL= number of larvae, LM= larval mass, ID=internal damage.
Means within columns followed by the same letters do not differ significantly at P=0.5, according to Duncan's multiple range test.

The frequency of distribution of the mass was established to help elucidate the differences in mass (mg) of larvae feeding in crosses (with inherited resistance) and in parental lines (susceptible local inbreds and two exotic resistant lines) (Tables 19 to 21).

4.4- Results and discussion

The results of the simple correlations between the three parameters of antibiosis (Tables 13 and 14) showed non-significant correlations between larval mass and leaf feeding damage in the F1 generations of both combinations. Most of the correlations were negative (Tables 13 and 14) and indicated that leaf feeding damage in the F1 hybrids was not affected by larval mass. This confirmed the result that more and less massive larvae were collected from plants of the F1 population, while in plants of the parental population larvae weighed much more (Tables 19 to 21) but were fewer in number. The increased number of larvae collected from F1 plants indicated that some chemical produced by the plant acted as an inhibitor to larval growth. The lack of larval stimulus to feed led to an increase in the number of larvae on the resistant plants (hybrids). The results provide an explanation for the highly significant correlation found between leaf feeding damage and larval mass in the parental plants (Table 14).

The frequency of distribution of larval mass are provided in Tables19 to 21. This illustrates the relationship between the larval mass and the numbers of surviving larvae in the two populations P and F1. The results show that in the parental population the 40% of all surviving larvae had a mass more than 200mg compared to only 10% in both

Table 13-Simple correlation between leaf feeding damage and larval mass in parental inbred lines and F1 hybrids resulting from crosses between resistant and susceptible inbred lines.

Inbred lines	Simple Correlation LM x LD		
	Parents	F1	
		xCML139	xMp706
P28	0.3985	0.0775	-0.3139
I137TN	0.3339	-0.3418	-0.2123
B73	0.7748**	-0.2134	-0.0349
B37	0.2581	0.1603	-0.2677
Mo17	0.7122**	-0.7089**	-0.1394
P608	0.5418	-0.2901	0.1334
F2834T	0.5387*	-0.3610	-0.3109
D5exp	0.9730**	0.0296	-0.2050
Oh43	0.9480**	-0.1608	-0.0330
Va35	0.9444**	0.1250	0.1105
Miacatlan	0.8901**	0.1931	-0.2821
K64R	0.8603**	-0.4345	-0.0482
P3	0.7271**	0.0952	0
P4	0.7107**	-0.0713	-0.2164
M37W	0.8895**	-0.1509	-0.1101
M162W	0.7238*	-0.2167	-0.0941
K0315Y	0.6968**	-0.0913	-0.2037
179 137TN	0.8949**	-0.0864	0
CML139	0.4605		
Mp706	0.4792		

LD= leaf damage; LM= larval mass.
*P=0.05, **P=0.01-level of significance based on t test.

Table 14-Simple correlation between larval mass and number of surviving larvae in parental inbred lines and F1 hybrids resulting from crosses between resistant and susceptible inbred lines.

Inbred lines	Simple Correlation LM x NL		
	Parents	F1	
		xCML139	xMp706
P28	0.5463	-0.0978	0.2761
I137TN	0.4427	-0.0106	0.4571*
B73	0.8067**	0.1716	0.6529**
B37	-0.059	-0.0511	0.0016
Mo17	0.4858	0.5518*	0.9141**
P608	0.7454**	0.5814*	0.0647
F2834T	0.0802	0.6128*	0.4664*
D5exp	0.5764*	0.3707	0.5126*
Oh43	0.5087	0.9692**	0.5577*
Va35	0.6068*	0.3894	0.4550
Miacatlan	0.6336*	0.8681**	0.4045
K64R	0.5973*	0.0759	0.3369
P3	0.1675	0.5837*	0
P4	0.7107**	0.3636	0.7513**
M37W	-0.1001	0.6338**	0.6184**
M162W	0.8265**	0.6112*	-0.0489
K0315Y	0.6241*	0.8366**	0.6543**
179 137TN	-0.2579	0.6911**	0
CML139	0.8065**		
Mp706	0.4773		

NL-number of larvae; LM- larval mass.
*P=0.05, **P=0.01-level of significance based on t test.

Table 15-Multiple regression coefficients and coefficients of determination for parental lines participating in crosses for insect resistance (P1) and (P2 resistants) CML139/*,Mp706/*.

Parental inbred lines	Regression Dependent Variable LM Y		Coefficient Independent Variables(X) X1(NL) X2(LD)	
	X1 (NL)	R-Squared	X2(LD)	R-Squared
P28	65.25 *	0.196	58.13 *	0.901
I 137TN	4.59	0.099	-7.61	0.010
B73	18.83 *	0.620	18.87	0.901
B37	0.39	0.001	11.71	0.077
Mo17	-25.07	0.319	28.96 *	0.579
P608	-5.22	0.003	2.56	0.002
F2834T	-60.04 *	0.317	17.23	0.139
D5Exp	-9.70	0.182	100.66 *	0.887
Oh43	-27.56 *	0.326	26.89 *	0.803
Va35	19.59 *	0.474	18.22 *	0.812
Miacatlan	-124.84 *	0.172	122.37 *	0.736
K64R	-68.32	0.130	71.41	0.189
P3	-13.60	0.001	35.72 *	0.549
P4	-2.15	0.064	34.56 *	0.489
M37W	1.98	0.016	50.96 *	0.445
M162W	35.12 *	0.758	62.18 *	0.938
K0315y	-41.43 *	0.183	52.77 *	0.460
179 I 137TN	96.23 *	0.682	12.56	0.171
CML139/*	-6.21	0.050	-34.31	0.216
Mp706/*	62.51 *	0.497	3.83	0.385

NL=Number of Larvae, LD= Leaf feeding damage, LM= Larvae mass.
R=Squared-Coefficient of Determination, X; Y= Dependent and Independent variables. *P=0.05.

Table 16- Multiple regression coefficient and coefficient of determination for the F1 Population from crosses of 18 local inbred lines with one insect resistance source (CML139).

F1 Hybrids	Regression Coefficient			
	Dependent Variable LM (y)		ndependent Variables (X) X1(NL) X2(LD)	
	X1 (NL)	R-Squared	X2(LD)	R-Squared
P28	-50.63 *	0.582	7.18	0.021
I 137TN	17.86 *	0.339	12.84	0.018
B73	5.17	0.022	-14.91	0.028
B37	75.50	0.059	-34.23	0.003
Mo17	7.59	0.005	-44.45	0.173
P608	29.76	0.874	1.47	0.064
F2834T	176.76	0.163	-227.09	0.045
D5Exp	27.35	0.268	52.20 *	0.068
Oh43	-26.43 *	0.364	-82.59 *	0.587
Va35	-39.62 *	0.441	-0.83	0.045
Miacatlan	-22.34	0.084	11.58	0.012
K64R	31.66	0.024	-121.75 *	0.492
P3	7.06	0.040	-19.06	0.009
P4	3.53 *	0.558	-21.65	0.013
M37W	12.20	0.190	-198.93 *	0.895
M162W	-8.47 *	0.070	-10.53	0.061
K0315y	-64.90	0.330	35.68	0.277
179 I 137TN	-9.01	0.059	1.52	0.006

NL=Number of Larvae, LD= Leaf feeding damage, LM= Larvae mass. R=Squared-Coefficient of Determination, X; Y= Dependent and Independent variables. *P=0.05.

Table 17-Multiple regression coefficients and coefficients of determination for the F1 population from crosses of 18 susceptible inbred lines with Mp706.

F1 Hybrids	Regression Dependent VariableLW (y)		Coefficient Independent Variables (X) X1(NL) X2(LD)	
	X1 (NL)	R-Squared	X2(LD)	R-Squared
P28	-23.01	0.051	-35.61	0.051
I 137TN	3.02	0.015	-8.77	0.085
B73	1.05	0.059	-6.69	0.208
B37	-37.64 *	0.522	-49.46 *	0.582
Mo17	32.58 *	0.736	-19.07 *	0.698
P608	-52.01	0.089	34.16	0.203
F2834T	-4.56	0.047	-0.68	0.047
D5Exp	-3.89	0.129	-28.52	0.238
Oh43	-16.14	0.916	-82.42 *	0.927
Va35	52.69	0.388	-14.06	0.301
Miacatlan	-6.80	0.149	-33.64	0.256
K64R	-34.76 *	0.392	-14.76	0.305
P3	0	0	0	0
P4	58.47	0.097	-64.02	0.098
M37W	10.74	0.099	-21.30	0.211
M162W	10.74	0.097	-21.31	0.098
K0315Y	-15.08	0.240	-6.41	0.132
179 137TN	-11.54	0.369	-48.87 *	0.448

NL=Number of Larvae, LD= Leaf feeding damage, LM= Larvae mass.
R=Squared-Coefficient of Determination, X; Y= Dependent and Independent variables. *P=0.05.

combinations of the F1. It can therefore be assumed that there is a direct relationship between the three parameters of antibiosis measured. There is a possibility for them to be inherited together. However, from field observations in segregating populations it appears that, depending on gene combinations inherited by a particular selection genotypes with numerous small holes in the leaves caused by larval feeding may yield high levels of resistance when crossed to those with few large holes.

The computed multiple regression provide some idea of how much changes occurs in any of the parameters LD and NL for a one-unit change in the larval mass LM (Tables15 to17).

4.5- Conclusion

The chemical nature of the antibiosis substance produced by the maize plant when attacked by *B. fusca is* unknown. The presence of such chemicals in genotypes affected by *B. fusca* was inferred by Van Rensburg (1993). Such chemicals are supposed to be 33Kda cysteine proteinase when plants are attacked by fall armyworm (Williams *et al.*, 1998; Chiang *et al.*, 2000).

Antibiosis assessment is difficult to apply in plant breeding for insect resistance since plants are to be destructed in order to determine larval survival. Its correlation with other parameters of resistance can be of use through chemical content evaluation (of which the amount can be used as an indicator of resistance) or by the use of molecular markers to tag the inheritance during the breeding program.

Table 19-Frequency distribution of mass of surviving *B. fusca* larvae, after 15 days feeding on 18 F1 crosses of susceptible inbred lines with one source of resistance (CML139).

inbred lines	N° of plants	Frequency Distribution mg											
		0 50	50 100	100 150	150 200	200 250	250 300	300 350	350 400	400 450	450 500	500 550	550 600
P28	17	10	6					1					
I137TN	17	8	1	5	2		1						
B73	17	7	7	3									
B37	17	5	5	3	1	1		1		1			
Mo17	17	9		2	4		1	1					
P608	17	7	3	3	1			1	1				1
F2834T	17	8	2	7									
D5Exp	17	5	2	4	4	2							
Oh43	17	10	2	4				1					
Va35	17	10	3		2		1		1				
Miacatlan	17	8	6	2		1							
K64R	17	14	2			1							
P3	17	17											
P4	17	11			1	1	1	2			1		
M37W	17	9	3	3	2								
M162W	17	1	1	3	2	4	4		1	1			
KO315Y	17	14	1		1	1							
179I137TN	17												

Table 20-Frequency distribution of mass of surviving *B. fusca* larvae, after 15 days feeding on 18 susceptible inbred lines (P1) and two resistant lines CML139 and Mp706 (P2) respectively.

inbred lines	N° of plants	Frequency Distribution mg											
		0 50	50 100	100 150	150 200	200 250	250 300	300 350	350 400	400 450	450 500	500 550	550 600
P28	9	2	1	1	4				1				
I137TN	13	2	2		2	6	1						
B73	11	3	4	2	1		1						
B37	13		1	1	5	2	2	1	1				
Mo17	14	6		1		1	1	2	2				1
P608	11	4			1	2		2	1	1			
F2834T	15	2			4	4		2		1	1	1	
D5Exp	14	5			2	3	2			1	1		
Oh43	6	3			1	1			1				
Va35	13	4		3									
Miacatl.	13	9	1	2		1							
K64R	14	6											
P3	11												
P4	15			2		1	2		2		1		
M37W	13	4	1		5	1	1						
M162W	13	8	2		1	1		1					
KO315Y	12		1	2	3	3	1	1	1				
179I137TN	15	3	2	3	2	3		1		1			
CML139	15	13			1		1						
Mp706	16	12	1	1	2								

P1- Susceptible parental lines, P2- Resistant parental lines.

Table 21-Frequency distribution of mass of surviving *B. fusca* larvae, after 15 days feeding on 18 F1 crosses of susceptible inbred lines with one source of resistance (Mp706).

Inbred lines	N° of plants	Frequency Distribution mg											
		0 50	50 100	100 150	150 200	200 250	250 300	300 350	350 400	400 450	450 500	500 550	550 600
P28	15	6	3	4		1	1						
I137TN	15	9	1	3	2								
B73	15	7	3	1	1		1	2					
B37	15	10	2	2	1		1	2					
Mo17	15	8	5	1	1								
P608	15	3	2		2	3	2	3					
F2834T	15	12		2	1								
D5Exp	17	4	6	6	1								
Oh43	16	12	2	2									
Va35	16	12	2	1					1				
Miacatl.	17	15	2										
K64R	13	8	3	1					1				
P3	13	8		1	1	2	1						
P4	17	8	3	3		1	1	1					
M37W	18	9	2	1	2	2	1		1				
M162W	16	6	7	2	1								
KO315Y	10	8		2									
179I137TN	15	12	1		1				1				

However, selection on the basis of antibiosis only can lead to the possible loss of others factors of resistance. So the use of this parameter in combination with other methods of assessment can be of great use in breeding programs.

Antibiosis as a chemical substance produced by the plant in presence of the insect is more regularly inherited and possibly transferred with more ease than other parameters of resistance It is assumed that non conventional methods such as markers may assist in the successful transfer of resistance to susceptible genotypes.

Chapter 5

The possible role of AFLP markers in breeding for maize resistant to *Busseola fusca*.

5.1- Abstract

Busseola fusca (Lepidoptera: Noctuidae) (Fuller) is an important pest in maize production in African countries South of Sahara. In this study we evaluated the different categories of assessment of leaf feeding resistance in maize to *B. fusca* using AFLPs. A total of 100 F2:3 recombinant inbred lines were produced from two cross combinations, inbred line P608 with two sources of resistance CML139 and Mp706 respectively. The tag of different fragments inherited in the same categories of resistance assessment in crosses of susceptible and resistant sources can be a good indicator of the inheritance of insect resistance. Ten categories of resistance were analysed by bulking five extracted plants sample of the F2:3 lines. The DNA of representing categories was studied using different primer combinations and as a result, several polymorphic fragments were detected in each of the progeny categories (resistant, intermediately resistant and susceptible) from both parental lines. The additive rather than dominant presence of the resistance related to the presence of AFLP fragments in this study.

Key words: *B. fusca* resistance, marker assisted selection, AFLP

5.2- Introduction

The use of genetic engineering and biotechnology holds great potential for plant breeding in terms of the time needed to introgress new traits into different crop varieties. It is expected that with the assistance of molecular markers, the transfer of insect resistance that is known to be polygenic can be facilitated. Molecular markers are specifically advantageous for agronomic traits that are otherwise difficult to tag (Mohan & Suresh, 1997).

Among the various markers developed until now, RFLPs were the first to be used in human genome mapping and later adopted for plant genome mapping (Weber & Helentjaris, 1989; Brown *et al.,* 1996).

Amplified fragment length polymorphism (AFLP) is based on PCR amplification of restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of a few nucleotide bases (Vos *et al.,* 1995). This technique was developed by Zabeau & Vos (1993) and Vos *et al.* (1995).

AFLPs are markers with a number of appealing features compared to RFLPs. Thus, they provide a novel and powerful tool for DNA fingerprinting of genomes of any origin or complexity including that of maize (Vos *et al.*, 1995).

A comparison of the three different DNA techniques RFLP, RAPD, and AFLP has been performed by Lin *et al.* (1996), for evaluation of their efficiency in detecting polymorphism in soybean. It was found that

AFLP is a most efficient technique in detecting polymorphism in soybean. High reproducibility, rapid generation and high frequency of identifiable AFLP polymorphism, makes AFLP analysis an attractive technique for identifying polymorphism and for determining linkages.

The use of RILs (recombinant inbred lines) in cultivars like *Pisum sativa* L and *Zea mays* L. has been reported by Mansur *et al.* (1993); Jinks (1981); Domoney *et al.* (1986); Burr *et al.* (1988); Carrilo *et al.* (1990) and Rousset *et al.* (1990). RI populations have additional recombination between linked loci and an increased power for detecting QTL (Cowen, 1988; Knapp & Bridges, 1990). The additional recombination should allow the resolution of some single QTL (in the F2:3) into multiple linked QTL (Austin & Lee, 1996).

The efficiency of a selection scheme or genetic analysis based on phenotype is a function of heritability of the trait factors like the environment, multigenic or quantitative inheritance or partial and complete dominance that often confound the expression of a genetic trait. Many of the constraints of a phenotypic-based assay can probably be mitigated through direct identification of genotypes with DNA-based diagnostic assay. For this reason DNA-based genetic markers are being integrated into several plant systems and are expected to play an important role in future insect breeding programs.

The increase in the number of publications with this purpose is a good indicator of the importance of this method (Asins *et al.,* 1988; Knapp *et al.,* 1990; Bentolila *et al.,* 1991; Edwards *et al.,* 1992; Dudley, 1992; 1993; Benson *et al.,* 1994; Khairallah *et al.,* 1994; Davarsi *et al.,* 1995;

Beavis & Smith 1996; Groh *et al.,* 1998). The aim of this study was to:

i- Evaluate the use of AFLPs for identification of *B. fusca* resistance in maize.

ii- Assess the relevance of a phenotypic method of breeding compared with MAS using AFLP markers.

5.3- Materials and methods

One of 18 susceptible maize inbred lines (P608) crossed with two exotic resistant lines was chosen to produce F2:3 lines for the AFLP analysis after the assessment of the resistance levels in the F2 generation of these crosses. Single seeds were randomly selected from F2 self-pollinated ears of P608 crossed with CML139 and Mp706. The ears were taken from each category in a range of one to 10, based on leaf feeding assessment of the F2 generation. Five ears per category were planted ear-to-row in a greenhouse trial. At the six-leaf stage of plant development, three leaves were collected from each of 10 plants per cross and lyophilized. This was used for DNA extraction, which increased the amount of extracted DNA up to 1000 samples (500 for each cross, named Lx1 and Lx2).

A total of 500 samples were analysed for each cross, Lx1 and Lx2. Lyophilized material was stored at -20°C until to be used for extraction.

5.4- DNA extraction

The DNA extraction was performed using a modified monocot method (Edwards *et al.*, 1991). Lyophilized leaves were ground to a fine powder in liquid nitrogen, after which 10ml of extraction buffer, (1M

Tris-HCl ph8; 0,25 M EDTA) and 20% SDS and 1ml Cetyl triethyl ammonium bromide (CTAB) was added. The homogenate was vortexed and incubated at 65°c for 60min. Thereafter cloroform-Isoamyl alcohol (24:1v/v) extractions were performed with centrifugation for 15 min at 10000 rpm. The DNA was precipitated by the addition of 100% ethanol. The precipitate was washed twice using 70% ethanol and centrifuged per 10 min at 15 000 rpm and finaly dissolved in 250µl of sterile distilled water. The concentration and quality of DNA was determined spectrophotometrically.

5.5- AFLP analysis.

AFLP analysis was done using bulk segregant analysis (BSA). The DNA from 10 plants was bulked to form a category sample. The AFLPs reactions were done according to the manufacturers' instructions (Gibco BRL).

5.6- Restriction endonuclease digestion and ligation of adaptors

Genomic DNA (250ng) was digested with Mse1 and EcoRI. The digested fragments were then ligated with EcoRI and Mse1 adapters (Table 22).

5.7-Polymerase chain reaction

A 51µl pre-selective PCR reaction was performed with 5µl diluted ligation product, pre-amp primer mix 10× PCR buffer and 1U of Ampli Taq DNA polymerase (GibcoBRL). A touchdown Hybaid thermal cycler

was used to perform the reaction for 20 cycles with the following profile: 30 s at 60 s at 56°C and at 60 s at 72°C. Pre-selective PCR products were diluted 50 fold in TE. Selective PCR-reactions were performed in a 20 μl PCR reaction containing 5μl of the diluted pre-selective reaction, 4.5μl of the Mse+3 primer (Table 22), 1μl Eco+3 (labelled) 2μl of 10×PCR buffer and 5U of Ampli Taq DNA polymerase. Reactions were performed for 36 cycles with the following cycle profile: at 30 s at 30 s at 65°C and a 60 s at 72°C. The 65°C annealing step was subsequently reduced by 0.7 °C for 12 cycles and then continued at 56 °C. A total of two primer combinations were tested.

After amplification 5μl of each of the selective reactions were added to 24μl of formamide and 1μl of Rox standard size marker, denatured at 94°C for 5 min and resolved on a Perkin Elmer ABI Prism 310 Automated capillary sequencer (PE Biosystems).

5.8- Results and discussion

A total of 266 AFLP fragments with primer combination Mse + CAC + EcoRI + ACA were identified of which 33 fragments (12.4%) were polymorphic. Polymorphisms were detected between the two parental lines and between the three groups of progeny categories.

The polymorphism between the parent lines is assumed to be a result of the genetic diversity distance between them. AFLPs fragment size ranged from 37 to 487 base pairs (bp), and the polymorphic fragments were distributed across all size ranges with major insidence for fragments between 137 to 400 bp.

Resistant parental lines had an average contribution to the phenotypic AFLPs fragments inherited by the progeny from the parents, of 9.4% of all inherited fragments (220). An average of 73 fragments (23%) were contributed by the susceptible parental line. The variability of the phenotypic diversity within the progeny categories is assumed to be additively contributed by the both parent fragments (36.5%). Fragments 145 and 190 were specifically inherited by the resistant categories and the intermediately resistant category. Their specific presence between the resistant parental fragments can be assumed to be donated to the progeny by the resistant parent. Fragment 233 was the only one specific simultaneously to the resistant progeny categories and the resistant parental lines. From all three fragments present in both resistant parental lines and the resistant and intermediately resistant progeny categories only fragment 233 can be reliable to tag the inheritance of the resistance, as fragments 145 and 190 were of low intensity.

Table 22 -A list of adapter and primer sequences used in AFLP

Mse-adapter	EcoR1-adapter
5'-GACGATGAGTCCTGAG-3'	5'-CTCGTAGACTGCGTACC-3'
Mse-primers (5'-GATGAGTCCTGAGTAA-3')	EcoR1-primers (5'-GATGCGTACCAATTC-3')
Mse + CAG	EcoRI +ACA (FAM)
Mse + CAG	EcoRI + AAC (NED)
Mse + CAC	EcoRI + ACA (FAM)
Mse + CAC	EcoRI + AAC (NED)

Categories 1, 2 and 3 had a major share of the total fragments inherited from the parental lines (40%, Table 23). Using the same primer combination (Mse + CAC + EcoRI + ACA) in the CML139 x P608 cross, 216 fragments were identified from which 84.7% were inherited by the nine categories from the parents. The susceptible parental line had a contribution of 53.2% to the total phenotypic diversity presented by the AFLP polymorphism. The additive share by the parents in the fragments present in all nine categories was 31.9% of the total fragments inherited by the progeny. The polymorphism was equaly present in all ranges of the fragments between the categories and between the two parental lines. Less polymorphism was found within near related progeny categories (NRC) such as 1, 2 and 3; 4, 5 and 6; 7, 8, 9 and 10 respectively (Table 24).

Using the second primer combination (Mse + CAG + EcoRI + ACA), to test the cross combination Mp706 x P608, 227 fragments of the size ranging from 75bp to 623bp were generated. Of the fragments 29.9% were distributed among resistant progeny, 25,5% to intermediately resistant and 25.8% among susceptible progeny categories. The parental lines had an average of 15.8% of all recorded fragments. The increase in size of the fragments increased the number of polymorphic fragments generated by this primer combination. Few polymorphic fragments were specific to selected progeny category such as it was with the fragment 433 for categories 1, 2, 3, 4 and the resistant parental line (Table 25).

In cross combination CML139 x P608, the primer combination Mse + CAG + EcoRI + ACA generated an average of 220 fragments with the

size ranging from 75 to 645bp. Several polymorphic fragments were detected in all 10 categories with 160 fragments being specific to categories 9, 10 and the susceptible parental line. Of the fragments, 26.8% were distributed between the three resistant progeny categories (Cat1, Cat2 and Cat3) of which eight were polymorphic (Table26). Most fragments were distributed among the susceptible progeny categories (31.4%). No specific fragment was found to characterize a specific category. The additive share by the two parents in all fragments inherited by the progeny was about 33.6% (74 fragments), where 51% (38 fragments) were located in the resistant progeny categories.

Using the primer combination Mse + CAG + EcoRI + AAC for the cross combination Mp706 x P608, few differences from the previous combinations were found. There was high similarity between the first three resistant progeny categories where of the 85 fragments detected in this group only 11.6% were polymorphic. However 300 fragments were generated by the use of this primer combination. Of these, 85% of the fragments were distributed among the progeny category with 28.3% to the resistant, 25.3% to the intermediately resistant and 31.3% to the susceptible progeny categories.

Table 23- Presence and absence of polymorphic fragments detected by the use of Mse + CAC +EcoRI + ACA primer combination by the use of AFLP markers in cross of susceptible and resistant to *B. fusca* inbred lines of maize (Mp 706 x P608).

Resistant Progeny			Intermediately resistant Progeny			Susceptible progeny			Parental lines	
Cat 1	Cat2	Cat3	Cat4	Cat5	Cat6	Cat7	Cat8	Cat9	Mp706	P608
37	37									37
66	66	66	66	66	66	66	66	66	66	66
71	71	71	71	71	71	71		71		71
74	74	74	74	74	74	74	74	74	74	74
85	85	85	85	85	85	85	85	85	85	85
97	97		97	97	97	97	97	97	97	97
103	103		103	103	103	103		103		103
121	121	121	121	121	121	121	121	121	121	121
126	126	126	126	126	126	126		126	126	126
137	137	137	137	137	137	137	137	137	137	137
145	145			145	145				145	
151	151		151	151	151					
155	155	155	155		155	155		155		155
					157	157		157		157
166	166	166	166		166	166	166	166		166
172	172	172	172	172		172		172	172	
			178	178	178	178	178	178	178	178
187	187				187	187				187
190	190		190	190					190	
196	196		196	196	196	196		196		196
202	202		202		202	202		202		202
208	208				208		208		208	208
212	212		212					212		212
221	221	221	221		221	221	221	221	221	221
233	233	233							233	
240	240	240	240			240		240		240
245	245	245	245	245	245		245	245	245	245
257	257	257	257		257	257		257	257	257
283	283	283	283			283		283	283	283
300	300	300				300	300	300	300	300
320	320	320	320	320		320		320	320	
366	366						366			
372	372		372							
400	400		400							
413	413		413			413		413		
444	444		444	444	444	444		444		444
474	474		474		474	474				
487	487					487	487			487

Mp706= resistant parent line. P608= Susceptible parent line.

Table 24- Presence and absence of polymorphic fragments detected by the use of Mse + CAC +EcoRI + ACA primer combination by the use of AFLP markers in cross of susceptible and resistant to *B. fusca* inbred lines of maize (CML139 x P608).

Resistant Progeny			Intermediately resistant progeny			Susceptible progeny			Parental lines	
Cat 1	Cat2	Cat3	Cat4	Cat5	Cat6	Cat7	Cat8	Cat9	CML139	P608
66	66	66	66	66	66	66	66	66		66
71	71	71	71	71	71		71	71		71
82	82	82	82	82	82	82	82	82	82	82
95	95	95	95	95	95	95	95	95	95	95
103	103	103	103	103	103	103	103	103		103
111	111	111	111	111		111	111	111	111	111
124	124	124	124	124	124	124	124	124		124
137	137	137	137	137	137	137	137	137	137	137
150	150	150	150	150	150		150	150		150
167	167	167	167	167	167		167	167		167
172		172	172	172		172	172	172	172	172
	180	180	180	180						180
	191	191		191	191		191	191		191
199	199	199	199	199	199			199		199
	202	202	202	202			202	202		202
209		208		208		208	208	208	208	208
222	222		222	222	222	222	222	222		222
245	245	245	245	245	245	245	245	245		245
257	257	257	257	257	257		257	257		257
278			278					278	278	278
		283	283	283		283	283	283		283
340		340		340			340			340
368		368		368			368	368		368
444	444	444	444	444	444	444	444	444	444	444
474	474	474	474	474	474	474	474	474		474

Mp706= resistant parent line.
P608= Susceptible parent line.

Table 25- Presence and absence of polymorphic fragments detected by the use of Mse + CAG +EcoRI + ACA primer combination by the use of AFLP markers in cross of susceptible and resistant to *B. fusca* inbred lines of maize (Mp706 x P608).

Resistant Progeny			Intermediately resistant Progeny			Susceptible progeny			Parental lines	
Cat 1	Cat2	Cat3	Cat4	Cat5	Cat6	Cat7	Cat8	Cat9	Mp706	P608
75	75		75		75		75	75	75	
89	89	89	89	89	89	89	89	89	89	89
104	104	104	104	104	104		104		104	
112	112	112	112		112	112	112	112		112
122	122	122	122	122	122	122	122	122	122	122
	126		126	126	126		126	126		126
170	170	170	170		170	170	170	170	170	170
184	184	184			184				184	184
210		210		210	210	210	210	210		210
237		237	237	237	237		237	237		237
244	244		244	244	244		244	244	244	
255		255	255	255	255	255	255	255	255	255
269	269	269	269	269	269		269	269		269
278	278	278	278	278	278		278	278		
299	299	299	299	299	299	299	299	299	299	299
318	318	318	318	318	318	318	318	318	318	318
335		335	335	335			335	335	335	335
356	356	356	356	356	356	356	356	356	356	356
385	385	385	385	385	385	385		385		
433	433	433	433					433	433	
452	452	452	452		452		452	452	452	
460	460	460	460	460	460		460	460	460	460
470	470	470	470	470	470	470	470	470	470	470
522	522	522	522	522	522			522	522	
	555		555		555	555	555	555	555	555
	623	623			623	623		623	623	

Mp706= resistant parent line.
P608= Susceptible parent line.

Table 26- Presence and absence of polymorphic fragments detected by the use of Mse +b CAG +EcoRI + AAC primer combination by the use of AFLP markers in cross of susceptible and resistant to *B. fusca* inbred lines of maize (Mp706 x P608).

Resistant Progeny			Intermediately resistant progeny			Susceptible progeny				Parental lines	
Cat 1	Cat2	Cat3	Cat4	Cat5	Cat6	Cat7	Cat8	Cat9	Cat10	Mp706	P608
50	50	50	50	50	50	50	50	50	50	50	50
56		56			56	56	56	56		56	56
69	69	69	69	69	69		69	69	69	69	69
73	73	73	73	73	73	73	73	73		73	
85	85	85	85	85	85	85	85	85		85	85
92	92	92					92	92	92	92	
100		100	100	100	100	100	100	100	100	100	100
107	107	107	107	107	107	107	107	107	107	107	107
114	114	114	114	114	114	114	114	114	114	114	114
125	125	125		125	125		125	125	125	125	125
128	128	128	128	128	128		128	128	128	128	
136	136								136		
144	144	144	144	144	144		144	144		144	
150	150	150	150	150	150	150				150	
160	160	160	160	160	160	160	160	160	160	160	160
170	170	170	170	170	170		170	170	170	170	170
200	200	200	200	200	200	200	200	200	200	200	200
212	212			212	212		212	212		212	
225	225	225	225	225	225	225		225	225		225
249	249	249	249	249	249	249	249	249	249	249	249
		275								275	
293	293	293	293	293	293	293	293	293	293	293	293
		300	300	300	300		300		300		300
326	326	326	326	326	326	326	326	326	326		326
340	340	340	340	340	340	340	340	340	340		340
350	350	350	350	350	350	350	350	350	350	350	350
400	400	400	400	400	400	400	400		400	400	
450	450	450	450	450	450	450	450	450	450	450	450
490	490	490	490			490		490	490		490
500	500	500	500	500	500	500	500	500	500	500	500
575	575	575			575			575			

Mp706= resistant parent line.
P608= Susceptible parent line.

Table 27- Presence and absence of polymorphic fragments detected by the use of Mse + CAG +EcoRI + AAC primer combination by the use of AFLP markers in cross of susceptible and resistant to *B. fusca* inbred lines of maize (Mp706 x P608).

Resistant Progeny			Intermediately resistant progeny			Susceptible progeny				Parental lines	
Cat 1	Cat2	Cat3	Cat4	Cat5	Cat6	Cat7	Cat8	Cat9	Cat10	Mp706	P608
50	50	50	50	50	50	50	50	50	50	50	50
56		56			56	56	56	56		56	56
69	69	69	69	69	69		69	69	69	69	69
73	73	73	73	73	73	73	73	73		73	
85	85	85	85	85	85	85	85	85		85	85
92	92	92					92	92	92	92	
100		100	100	100	100	100	100	100	100	100	100
107	107	107	107	107	107	107	107	107	107	107	107
114	114	114	114	114	114	114	114	114	114	114	114
125	125	125		125	125		125	125	125	125	125
128	128	128	128	128	128		128	128	128	128	
136	136								136		
144	144	144	144	144	144		144	144		144	
150	150	150	150	150	150	150				150	
160	160	160	160	160	160	160	160	160	160	160	160
170	170	170	170	170	170		170	170	170	170	170
200	200	200	200	200	200	200	200	200	200	200	200
212	212			212	212		212	212		212	
225	225	225	225	225	225	225		225	225		225
249	249	249	249	249	249	249	249	249	249	249	249
		275								275	
293	293	293	293	293	293	293	293	293	293	293	293
		300	300	300	300		300		300		300
326	326	326	326	326	326	326	326	326	326		326
340	340	340	340	340	340	340	340	340	340		340
350	350	350	350	350	350	350	350	350	350	350	350
400	400	400	400	400	400	400	400		400	400	
450	450	450	450	450	450	450	450	450	450	450	450
490	490	490	490			490		490	490		490
500	500	500	500	500	500	500	500	500	500	500	500
575	575	575			575			575			

Mp706= resistant parent line.
P608= Susceptible parent line.

It was found that the share of the fragments from the different progeny categories of phenotypic assessment of resistance gained in crosses of one susceptible inbred line with two different sources of resistance, indicated the predominance of the fragments with additive contribution of the two parents. The reliability of the values of specific fragments to indicate a specific kind of resistance have to be demostrated in the future conducting other experiments using this specific primer combination. The differences found using different primer combinations for the two different cross combinations indicated that models to identify progenies with inherited resistance can be established for each specific cross combination between susceptible and resistant sources.

5.9- Conclusions

The segregation analyses have shown that AFLP markers are inherited in a Mendelian manner (Maughan *et al.,* 1996). From this point of view it is possible to assume that the generated fragments by the use of AFLP markers can be of use to determine the inheritance of *B. fusca* resistance from the crosses of resistant and susceptible inbreds. A great number of additive fragments, up to 56%, found to be inherited by the progeny from the two parental lines was an indication that there is more than one locus participating in the resistance. The low heritability of the fragments with resistance was similarly found by the phenotypic evaluation of the crosses. It can be assumed that an increase of the phenotypic heritability will also be linked to an increase in the presence of fragments specifically related to the resistance source. Distribution of the fragments for the two different cross

combinations have proved the assumption that the resistance is inherited diferently by the susceptible lines related to the kind of reisistance source to be used. Differences in amplified fragment-length polymorphisms were also studied in fall armyworm (McMichael & Prowell, 1999).

It was concluded that different sources of insect resistance donate different fragments to the susceptible lines. An evaluation of the fragments that are specific to certain sources of resistance and are inherited by the susceptible lines will help the breeding program for insect resistance in saving time to identify the suitable lines to be combined with certain sources of resistance with success.

Chapter 6

General conclusions

The increase of maize production is seen in Africa not only as a source of financial profits but essentially as a possibility of relief from poverty and hunger.

The 10% reduction in the global maize production in South Africa and much more in other African countries caused by insect attacks should be seen as a great challenge for all maize breeders. With this study we intended to accumulate knowledge about the nature of the inheritance of *B. fusca* resistance in maize.

Susceptible inbred lines were crossed with two different sources of resistance. Phenotypic and AFLP screening was conducted in the field and under laboratory conditions to estimate the gains of the resistance by the progeny from the crosses. The results of this study indicated that the study of the inheritance of the insect resistance to *B. fusca* couldn't be effective by using conventional methods of breeding alone.

The following questions have been found to complicate the study using conventional procedures: 1-the used methods of evaluation of the leaf feeding are never very reliable because they are based on pure phenotypic expression of the character. This expression of the

insect resistance is associated with non-heritable parameters (epistasis) that, in the early generations of crossed material masks the real image of the phenotype that complicate the selection. 2- Backrossing is the procedure that should allow the breeders to fix the gained gene effects from the crosses, but backcrossing is difficult because of the polygenic nature of the insect resistance where the genes responsible for the resistance are located on different loci (no linkage).

The results of the AFLP marker study indicated the importance of the use of this tool to help understand the nature of the insect resistance, but more experiments should be conducted with more primer combinations and different cross combinations.

The combined use of the phenotypic and marker based selection will be of great importance to the future studies of the inheritance of the insect resistance.

Chapter 7

Summary

The stem borer *Busseola fusca* (Lepidoptera: Noctuidae) is an important pest in maize production in South Africa and many other countries South of the Sahara.

The mean goal of the present study was to gather information about the genetic and phenotypic characteristics of *B. fusca* resistance in maize as a help to understand the nature of the resistance in order to overcome the difficulties standing in the way of transfer of the resistance from available sources to the local and adapted cultivars.

The inheritance was studied through assessment of the resistance on basis of phenotypic expression after artificial infestation with first instar larvae of *B. fusca* of 36 crosses of 18 susceptible inbred lines with two sources of resistance, CML139 and Mp706. Plants were evaluated for characters like leaf feeding, larval mass gain, internal damage and yield losses. A scaling test was used to analyse the data. Results indicated that additive, dominant and non-heritable parameters were all important for *B. fusca* resistance. GCA and SCA values indicated good performance of the crosses for additive and dominant gene effects (heterosis). The correlation coefficient was used to evaluate the relatedness of three parameters of assessment of the resistance and the result indicated that there are significant correlations between leaf feeding, larval mass gain and internal damages caused by the insect.

Different levels of inheritance from the two sources were seen in each cross indicating that the resistance is inherited differently, depending on the source used.

F2:3 lines were obtained from selected crosses (CML139 xP608) and (Mp706 xP608) for AFLP analysis. The analysis of the 10 categories of phenotypic assessment evaluated for the fragment segregation indicated that additive gene contribution from the parents was present at several loci. This was in agreement with negligible one locus non allelic interaction found by the scaling test for absence of epistasis. Different fragments were found to be specific for resistant parents and the progeny, which indicated that dominance was again present in the inheritance of the resistance.

In this study we have concluded that despite attempts to improve the varieties' resistance by transfer of the resistance from resistant sources to more adapted varieties, the polygenic nature of the resistance and the presence of high levels of non-inherited parameters are still the most important cause of ineffective use of conventional methods of breeding.

The use of markers to tag the genetic information about the inheritance of the resistance in cross progenies of resistant and susceptible varieties is seen as one of the ways to overcome this barrier. Unfortunately the use of this important tool is still not perfected for use in this particular area of science. Until the perfection of molecular marker technology, the recurrent selection approach will have to be used for insect resistance improvement in maize cultivars.

Opsomming

Die stamboorder *Busseola fusca* (Lepidoptera: Noctuidae) is 'n belangrike insekplaag in mielie produksie in Suid Afrika en heelwat ander lande suid van die Sahara.

Die doel van hierdie studie was om inligting in te samel van genetiese en fenotipiese eienskappe van *Busseola fusca* weerstand in mielies as 'n metode om die aard van die weerstand te verstaan, om oordrag van weerstand van weerstandsbronne na plaaslike en aangepaste cultivars te vergemaklik.

Die oorerflikheid is bestudeer deur evaluasie van weerstand op die basis van fenotipiese uitdrukking na kunsmatige infestasie met eerste instar larwes van *Busseola fusca* in 36 kruisings van 18 vatbare ingeteelde lyne met twee weerstands bronne CML139 en Mp706. Plante is geëvalueer vir eienskappe soos blaar voeding, larwes se massa toename, interne skade en opbrengs verliese. Resultate het aangetoon dat additiewe, dominant en nie-oorerflike parameters almal belangrik was vir *Busseola fusca* weerstand. GCA en SCA waardes het goeie additiewe en dominante geen aksie (heterose potensiaal) van kruisings aangetoon. Korrelasies is gebruik om verwantskappe tussen drie parameters van weerstands evaluasie te bepaal. Die resultate het aangetoon dat daar betekenisvolle korrelasies is tussen blaar voeding, larwes se massa toename en interne skade veroorsaak deur die insek.

Verskillende vlakke van oorerwing van die twee weerstandsbronne was duidelik in elke kruising, wat aantoon dat weerstand verskillend oorgeërf word afhangend van die weerstandsbron wat gebruik is. F2:3 lyne is gekry vanaf geselekteerde kruisings (CML139xP608 en Mp706xP608) vir AFLP analise. Die analise van die 10 kategorië van fenotipiese evaluasie wat gebruik is vir fragment skeiding het aangedui dat die additiewe bydrae van die ouers teenwoordig was by verskillende loci, wat in ooreenstemming was met die weglaatbare een lokus nie-alleliese interaksie wat gevind is in die skaal toets vir afwesigheid van epistase. Verskillende fragmente is gevind wat spesifiek was vir weerstandbiedende ouers en nageslag, wat aangedui het dat dominansie weereens teenwoordig was by oorerwing van weerstand.

In hierdie studie is die gevolgtrekkings gemaak dat ten spyte van die poging om cultivars se weerstand met oordrag van weerstandbronne na aangepaste cultivars te verbeter, die poligeniese aard van weerstand en die hoë vlakke van nie oorerflike parameters die grootste oorsaak is van oneffektiewe gebruik van konvensionele teeltegnieke.

Die gebruik van merkers om genetiese inligting te merk van oorerwing van weerstand in die kruisings nageslag van weerstandbiedende en vatbare ouers is een manier om hierdie probleem te oorkom. Ongelukkig is die gebruik van hierdie tegnologie nog nie perfek vir hierdie gebied in die wetenskap nie. Totdat die tegnologie vervolmaak is, sal die herhalende seleksie benadering nog gebruik moet word vir verbetering van insek weerstand in mielies.

Chapter 8

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