Ramesh Arora · Surinder Sandhu

Editors

Breeding Insect Resistant Crops for Sustainable Agriculture

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Ramesh Arora
Department of Entomology & Zoology
Eternal University
Baru Sahib, Himachal Pradesh, India

Surinder Sandhu
Department of Plant Breeding and Genetics
Punjab Agricultural University
Ludhiana, Punjab, India

Preface

The world population has been galloping upward at an unprecedented rate in the recent past and has jumped from 3.5 billion to more than 7.4 billion during the last 50 years. So far, modern agricultural technologies have enabled us to meet the rising demand for food, feed, and fiber for the increasing human population through improved productively of major crops. But modern crop protection practices, based largely on the intensive use of pesticides, have failed to reduce crop losses by insect-pests, which still destroy an estimated one-fifth of the global agricultural production of important crops. Rather, pesticidal interventions in the agroecosystem have created human health hazards, lowered environmental quality, and disrupted natural control of pests. Therefore, there is an urgent need to strengthen non-chemical approaches for reducing pest damage, which should be safe, economical, and durable.

Pest-resistant cultivars represent one of the most environmentally benign, economically viable, and ecologically sustainable options for utilization in pest management programs. Beginning in the 1920s, modern work on plant resistance to insects was pioneered by Professor R. H. Painter and colleagues at Kansas State University, USA. This paved the way for notable successes in developing pest- and disease-resistant cultivars. Hundreds of insect-resistant cultivars of rice, wheat, maize, sorghum, cotton, sugarcane, and other crops have been developed worldwide and are grown extensively for increasing and stabilizing crop productivity. Remarkable success was achieved in developing multiple pest- and disease-resistant rice cultivars especially IR-36, IR-64, IR-72, and IR-74 by Professor G. S. Khush and colleagues at the International Rice Research Institute, Los Banos, Philippines. The wide adoption of these cultivars led to a quantum jump in rice production in tropical Asia. Similar but less spectacular successes were also achieved in several other important crops. As per recent estimates, the annual economic value of arthropod resistance genes deployed in global agriculture is greater than US$2 billion.

Despite spectacular achievements and even greater potential for contributing to sustainable agriculture, only a handful of books have been published on the topic of host-plant resistance to insects. Professor R. H. Painter published his monumental book *Insect Resistance in Crop Plants* (MacMillan) way back in 1951 and laid the foundations of HPR to insects as a sub-discipline in agricultural entomology and crop protection. Other major works include *Plant Resistance to Insects: A Fundamental Approach* (Wiley) by C. Michael Smith (1989), *Host Plant Resistance to Insects* (CABI) by N. Panda and G. S. Khush (1995), and *Plant Resistance to*

Arthropods: Molecular and Conventional Approaches (Springer) by C. Michael Smith (2005).

The advent of molecular biology tools has enabled us to overcome some of the major limitations of conventional breeding approaches. The new book *Breeding Insect Resistant Crops for Sustainable Agriculture* emphasizes the recent advances in host-plant resistance to insects, which have enhanced our capability and speed to develop insect-resistant cultivars for improving productivity as well as for bringing stability in agricultural production.

The introductory chapter by the two editors gives an overview of the fascinating science of insect-plant interrelationships, which provides the bases for development of insect-resistant crop plants. The second chapter provides a concise account of the extent of losses caused by insect-pests in important crops. The commercial cultivation of insect-resistant cultivars can help in minimizing these losses in an environmentally benign manner. The breeding methods for developing insect resistance in self- and cross-pollinated crops have been elaborated in Chap. 3. The new insights on structural and functional aspects of insect resistance conferring R-genes have been emphasized for their better utilization by researchers.

Leaf hoppers and plant hoppers are major biotic constraints in rice production, and consistent research efforts on HPR to hoppers have resulted in identification of more than 70 genes for resistance to hoppers. Several hopper-resistant rice cultivars are being grown commercially around the world, and their development, status, and prospects are reviewed in Chap. 4. Several species of insect-pests limit the production and productivity of grain legumes, which are major dietary sources of proteins for the humans. The success, limitations, and prospects of development of insect-pest-resistant genotypes of grain legumes have been reviewed in Chap. 5. The productivity of oilseed brassicas is severely affected by aphid pests, but not much progress has been made in breeding for resistance in brassicas against aphids primarily due to nonavailability of resistance source within the crossable germplasm as well as lack of knowledge on its trait genetics. The problems and prospects for development of aphid resistance in brassicas are enumerated in Chap. 6.

Maize, being a leading contributor to the world cereal basket, has undergone various improvements through diverse breeding tools to minimize the losses due to insect-pests. Chapter 7 provides an overview of these efforts including the application of novel breeding methods for development of insect-resistant cultivars of maize. Sorghum and millets are crucial to the food and nutritional security in arid and semiarid regions of the world. Considerable success has been achieved in developing sorghum and millets genotypes resistant to shoot fly and to a lesser extent to stem borer and other pests. The progress, problems, and prospects for incorporating insect-pest resistance in sorghum and millets are outlined in Chap. 8. Cotton crop suffers from ravages by a wide range of insect-pests and has received a lot of attention for nearly a century for incorporating resistance to sucking pests as well as bollworms using conventional and molecular techniques. The development of insect resistance in cotton is described in Chap. 9.

The development of insect-resistant cultivars of fruit plants provides a durable alternative to the use of insecticides for management of insect-pests. The classical breeding

approaches have been complimented with innovative biotechnological tools to achieve the desired results as discussed in Chap. 10. The status of development of rice geno-types resistant to stem borers and gall midge presents two contrasting scenarios as illustrated in Chap. 11. The sources for gall midge resistance available in crossable gene pool have been exploited to produce gall midge-resistant cultivars, which have been released for commercial cultivation. But, due to a lack of sources of resistance against stem borers, the alternate approaches like Bt-transgenics and RNAi are being pursued for development of borer-resistant rice.

Chapter 12 outlines the sources of resistance available for major insect-pests of mung bean and urd bean, mechanism of resistance, and current status as well as prospects for development of insect-resistant cultivars in these crops. Insects being versatile organisms can overcome plant resistance by developing new biotypes, which adversely affect the sustainability and durability of insect-resistant cultivars. The evolution of insect biotypes and strategies for their management are outlined in the concluding chapter.

We are thankful to all the contributors for the meticulous job they have done in preparing their respective chapters. Special thanks are due to Professor M. S. Kang, formerly vice-chancellor at Punjab Agricultural University, Ludhiana, for guiding us throughout the preparation of this manuscript. It is hoped that the book will fill the wide gap in literature on breeding for insect resistance in crops. It is intended for plant breeders, entomologists, plant biotechnologists, and IPM experts, as well as those working on sustainable agriculture and food security.

Baru Sahib, Himachal Pradesh, India   Ramesh Arora
Ludhiana, Punjab, India   Surinder Sandhu

Contents

Contributors

P.K. Arora Regional Research Station, Punjab Agricultural University, Abohar, India

Ramesh Arora Department of Entomology & Zoology, Eternal University, Baru Sahib, Himachal Pradesh, India

Department of Entomology, Punjab Agricultural university, Ludhiana, India

S.S. Banga Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

J.S. Bentur Directorate of Rice Research, Hyderabad, India

Vitthal R. Bhagwat ICAR-Indian Institute of Millets Research, Hyderabad, India

Dharminder Bhatia Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

D.S. Brar School of Agricultural biotechnology, Punjab Agricultural University, Ludhiana, India

Plant Breeding, Genetics, and Biotechnology Division, International Rice Research Institute, Los Baños, Philippines

Harpreet Kaur Cheema Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

M.I.S. Gill Department of Fruit Science, Punjab Agricultural University, Ludhiana, India

Jagdish Jaba International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India

Manjit S. Kang Department of Plant Pathology (Geneticist), Kansas State University, Manhattan, KS, USA

Punjab Agricultural University, Ludhiana, India

Chikkappa G. Karjagi Indian Institute of Maize Research, New Delhi, India

Sanjeev Kumar Kataria Punjab Agricultural University, Bathinda, India

Jaswinder Kaur Indian Institute of Maize Research, New Delhi, India

Rubaljot Kooner Department of Entomology, Punjab Agricultural University, Ludhiana, India

Krishan Kumar Regional Research Station, Punjab Agriculture University, Abohar, India

Pradyumn Kumar Indian Institute of Maize Research, New Delhi, India

Sarwan Kumar Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

Soujanya P. Lakshmi Indian Institute of Maize Research, New Delhi, India

Gurpreet Singh Makkar Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

M.G. Mallikarjuna Indian Institute of Maize Research, Pusa Campus, New Delhi, India

Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Aditya Pratap ICAR-Indian Institute of Pulses Research, Kanpur, India

P. Sanjana Reddy ICAR-Indian Institute of Millets Research, Hyderabad, India

Surinder Sandhu Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

P.S. Sarao Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana, India

J.C. Sekhar Indian Institute of Maize Research, New Delhi, India

H.C. Sharma Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, India

International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India

Smriti Sharma Department of Entomology, Punjab Agricultural University, Ludhiana, India

G. Shyam Prasad ICAR-Indian Institute of Millets Research, Hyderabad, India

Paramjit Singh Punjab Agricultural University, Bathinda, India

S.B. Suby Indian Institute of Maize Research, New Delhi, India

G.K. Sujayanand ICAR-Indian Institute of Pulses Research, Kanpur, India

Gaurav K. Taggar Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana, India

Vilas A. Tonapi ICAR-Indian Institute of Millets Research, Hyderabad, India

Sumit Vashisth International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India

About the Editors

Dr. Ramesh Arora is professor of entomology at Eternal University, Baru Sahib, HP, India, and holds a Ph.D. in entomology (1990) from Punjab Agricultural University (PAU), Ludhiana, Punjab, India. He served at PAU for more than 37 years as a researcher in various capacities. His research interests include development and dissemination of IPM in cotton, vegetables, oilseeds, and forage crops and climate-resilient pest management. Dr. Arora has nearly 300 publications, including 90 research articles in refereed national and international journals and 100 conference/symposia presentations. He has also authored/edited 14 books on entomology, pest management, and sustainable agriculture and contributed 42 book chapters in edited books published by leading publishers. He has been involved in designing course curricula and teaching UG and PG courses in entomology. He has guided the research work of more than 30 PG students in entomology, plant pathology, zoology, microbiology, biochemistry, agricultural biotechnology, and extension education. Dr. Arora is associated with several professional societies as vice president/general secretary/editor in chief/EC member and has served as organizing secretary/co-organizing secretary/member of the organizing committee of more than a dozen international and national conferences/seminars/symposia.

Dr. Surinder Sandhu is professor of plant breeding at Punjab Agricultural University (PAU), Ludhiana, Punjab. She received her Ph.D. in genetics (minor in plant breeding) from PAU, Ludhiana, in 1995 and won the prestigious J. L. Nehru Award for outstanding Post Graduate Research in Agriculture from ICAR, New Delhi. She started her research career as scientist (crop improvement) (1995–2002) at the ICAR-Central Potato Research Institute's Regional Station, Jalandhar. Later, she became a sugarcane breeder (2002–2010) at PAU, Ludhiana. She served as a research scholar at the University of Florida, Gainesville, USA, from December 2007 to June 2008. She received the Best Teacher Award (ICAR) for the year 2010–2011 and was granted the Australian Endeavour Research Fellowship Award in 2010. Dr. Sandhu has been instrumental in the development of four sugarcane varieties, which were released in the state, two of which were recommended at zonal level. She has published 24 research papers in refereed international and national journals and written three manuals and four book chapters. She was promoted to the position of professor of plant breeding in 2011 and presently working on varietal develoment in oilseed crops.

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Insect-Plant Interrelationships

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Ramesh Arora and Surinder Sandhu

Abstract

The green plants and insects represent the two dominant groups of living organisms on Earth. The green plants occupy the most capacious segment among all biological organisms, whereas the insects are the most specious group. These two 'empires' are interconnected as well as interdependent. Green plants are the primary producers of food, and all animals being heterotrophs depend directly or indirectly on plant-produced food. In turn, nearly three fourths of all angiosperms require the services of insect pollinators. The entomophilic flowering plants and their insect pollinators thus represent the most evident and widely applicable example of mutualism among living organisms. But a wide variety of phytophagous insects also flourishes, diversifies and sustains on these plants. Consequently, the plants have evolved a dizzying array of morphological and biochemical (constitutive as well as induced) barriers for protection against insects and other herbivores. Evolutionary interactions between plants and insects may have contributed to the increased biodiversity and success of both these groups. The study of these interrelationships, as outlined in this chapter, is of great practical significance for the future agricultural production. The development of pest-resistant cultivars of crop plants and progress in integrated pest management both require an intricate understanding of insect-plant relationships.

R. Arora (✉)
Department of Entomology & Zoology, Eternal University,
Baru Sahib, Himachal Pradesh 173101, India
e-mail: arorarame@gmail.com

S. Sandhu
Department of Plant Breeding and Genetics, Punjab Agricultural University,
Ludhiana, Punjab 141 004, India
e-mail: surindersandhu@pau.edu

State-of-the-art techniques such as mutant analysis, metabolomics, RNAi and proteomics developed during the last three decades have been instrumental in providing improved insight into these interrelationships.

1.1 Introduction

The 'plant kingdom' and the 'class Insecta' represent the two dominant groups of living organisms, in terms of the abundance of species as well as in the amount of biomass. Green plants are the primary producers of food, and all animals being heterotrophs depend directly or indirectly on plant-produced food (Schoonhoven et al. 2005). In turn, a majority of the 300,000 plant species require the services of insect pollinators for reproduction. Colourful, scented flowers and floral nectarines were in all probability developed by plants for attracting insect pollinators. Flower anatomy ensured that while feeding, the insects also picked up the pollen (Kearns et al. 1998). Consequently, to prevent over-exploitation, the plants have also evolved a dizzying array of structural and biochemical barriers for protection against insects and other herbivores. While some of these barriers are synthesized by plants regardless of the presence of herbivores (constitutive defences), many others are produced only in response to herbivory (induced defences). Only those insect species, which are able to overcome these barriers in one or more plant species by avoidance, detoxification, etc., can access that plant species as food. The insects which damage the economically important plants have been termed as 'insect pests' by humans. The important mutualistic and antagonistic interactions between plants and insects are introduced hereunder.

1.2 Mutualistic Interactions: Flowering Plants-Insect Pollinators

The most evident and widely applicable example of mutualism is that between insect-pollinated flowering plants and their insect pollinators. Nearly 80% of all flowering plants are bisexual and bear flowers with stamen and pistils in the same flower. This promotes self-fertilization and consequently inbreeding. The plants avoid self-fertilization either by separating the sexes in time and space (differences in the timing of maturation) or by self-incompatibility. Both mechanisms promote cross-pollination, which is assisted by various agencies e.g. wind, water, and animals, etc. More than three fourths of all flowering plants are wholly or partially

insect-pollinated (Faegri and Pijl 1971). The economic value of insect pollinators is enormous. Most of the important oilseeds, pulses, fruits, vegetables, nuts, spices and ornamentals (Hill 1997; Atwal 2000) show improved yields with animal pollination (Klein et al. 2007). It has been estimated that animal pollination has an economic value of €153 billion annually, which is nearly one tenth of global agricultural production (Galai et al. 2009).

Some of the widely accepted estimates of the number of angiosperms pollinated by animals vary from 67% to 96% of all angiosperm species (Axelrod 1960; Nabhan and Buchmann 1997). Ollerton et al. (2011) observed that these estimates are not based on firm data. They compiled data on published and unpublished community level surveys of plant-pollinator interactions and concluded that proportion of animal-pollinated species was 78% in temperate-zone communities and 94% in tropical communities, with a global mean of 87.5% of all flowering plants. The pollinators benefit from rewards in the form of nectar and pollen. Both are nutrient-rich foods with nectar containing 50% sugars and pollen 15–60% proteins and other essential elements (Proctor et al. 1996; Roulston et al. 2000). Together, they provide nourishment for the bees, which are the most important among insect pollinators (Schoonhoven et al. 2005).

The entomophilous flowering plants and the pollinating insects constitute an example par excellence of mutualism. However, the degree of mutualism varies among various plant-pollinator combinations (Schoonhoven et al. 2005). In some cases, there is obligate mutualism, and a species of plant can only be pollinated by a single species of pollinator, which depends on it for food. For instance, figs (*Ficus* spp., Moraceae) are dependent upon fig wasps (Agaonidae, Chalcidoidea) for pollination (Wiebes 1979). Every species of fig is pollinated by a specific wasp species, e.g. the pollination in *Ficus carica* Linnaeus is carried out by the fig wasp, *Blastophaga psenes* (Linnaeus) (Ramirez 1970). Another example of obligate mutualism is observed between yucca moths (Prodoxidae) and yucca plants (Agavaceae). The yucca moths are the sole pollinators for yucca flowers and deposit their eggs in the locule of the ovary of flowers so that the young caterpillars can feed on the developing seeds (Pellmyr and Krenn 2002).

Another interesting example is based on the great naturalist Charles Darwin's prediction. In 1862, while doing research on orchids, Darwin found that the astounding Christmas orchid, *Angraecum sesquipedale* Thouars, had nearly a foot-long green nectary. As this group of orchids was moth pollinated, Darwin predicted that there must be a gigantic moth species with extended proboscis capable of feeding on the long nectary. More than four decades later, Rothshild and Jordan in 1903 described the Morgan's sphinx moth, *Xanthopan morganii* Walker with an extended proboscis length of >12 in., as the only known pollinator of *A. sesquipedale*, which is endemic to Madagascar (Kritsky 2001). However, such reciprocal evolution in plant-pollinator relationships is not widespread. Burkle and Alarcon (2011) observed that most plant-pollinator relationships have a fairly broad range with a high degree of annual turnover of pollinator species, and the relative importance

of a pollinator species may vary in different years for pollination of the same plant species.

Insect pollination has undoubtedly contributed to the evolutionary success of angiosperms. The fossil records show that pollination originated around 250 Myr ago (Labandeira 2013). The early angiosperms were probably pollenized both by the wind and animals. In view of the advantages conferred by entomophily, its importance increased over evolutionary time (Cox 1991; Crepet et al. 1991). Entomophilic angiosperms display a diversity of flower size, shape, colour and fragrance which may have been determined by the requirements of the pollinators. The pollen in flowers of such plants may have a sculptured structure and/or is covered with sticky substances which help it to easily adhere to the insect body. The hairs on the insect legs and other body parts also aid in pollen transfer. The bumble bee pollinated flowers in foxglove, *Digitalis purpurea* Linnaeus are bell shaped, while the butterfly pollinated flowers of *Calopheria* spp. have tubular corolla, which is an adaptation to the long probocis (Schoonhoven et al. 2005). In addition, the latter contain higher levels of amino acids than flowers fed on by flies (Baker and Baker 1986). In order to attract pollinators, some plant species produce sterile 'reward anthers' which are brightly coloured (Nepi et al. 2003). Flowers of the orchid Mirror of Venus, *Ophrys speculum* Link, imitate the virgin female wasps of their pollinator, *Dasyscolia ciliata* (Fabricius), by releasing the female sex pheromone to entice the male wasps. The attracted male wasps try to mate with the flowers and in doing so act as pollination vectors (Ayasse et al. 2003).

Hymenopterans, especially the Apoidea, are the most important group involved in flower pollination at present, but other groups have been equally important in the past. Basal angiosperms are even now primarily pollenized by the beetles and flies (Thien et al. 2000). Bees are closely adapted to a floral diet (Atwal 2000) and are able to assimilate pollen grains despite the presence of an almost impermeable cuticle (Velthius 1992). Individual honeybees often exhibit flower constancy by preferably visiting flowers of a single species. It improves pollinator efficiency and also helps in reproductive isolation of plant species. The insects' ability to remember combinations of flower odours and colours plays a central role in flower constancy. Honeybees have been reported to have the capacity to distinguish at least 700 different floral aromas (Schoonhoven et al. 2005).

1.3 Antagonistic Interactions: Herbivorous Insects-Green Plants

Insects are the most diverse and a tremendously successful group of organisms on Earth. The members of a number of insect orders infest plants and obtain food from them. Species in some of the insect orders are almost exclusively (Lepidoptera, Orthoptera, Phasmida) or predominantly (Hemiptera, Thysanoptera) herbivorous. But Coleoptera, Hymenoptera and Diptera are only partly herbivorous and also include numerous carnivorous species (Schoonhoven et al. 2005). Every vascular

plant species usually harbours several insect species. There are insect species feeding on all parts of the plant including the roots, stem, bark, shoots, leaves, flowers and fruits. While solid feeders chew plant tissues externally (defoliators) or internally (borers), others suck the sap (aphids, jassids), reduce plant vigour and even act as vectors of plant pathogens, e.g. whitefly.

Most insects usually exhibit a high degree of specialization in their choice of food plants. The monophagous insects feed on only a single or a few closely related species of plants, while oligophagous ones feed on a number of plant species, all of which belong to the same family. In contrast, the polyphagous insects use a wide range of plants from different plant families as food (Panda and Khush 1995). But most insects exhibit some degree of specialization in their host plant choice. Investigation on herbivorous insects has revealed that only around one tenth of these insects have the ability to feed on plants of more than three plant families. The host range of each insect species is constrained by several structural, biochemical and ecological factors. As a generalization, it may be stated that, except for Orthoptera, all other orders of herbivorous insects are largely composed of species specialized to feed on particular plant species (Schoonhoven et al. 2005). According to Bruce (2015), the herbivores have evolved over time to become specialized feeders, even though some of polyphages continue to be important agricultural pests. Insects have the ability to recognize and respond to host cues for feeding and oviposition.

Despite the antagonistic relationships between plants and phytophagous insects presumed to operate in all cases, herbivory has been observed to increase plant growth and fitness in some cases (Owen 1980; Vail 1994; Sadras and Felton 2010). Yield decreases due to arthropod feeding are quite common, but there are examples of increased yield recorded in insect-damaged as compared to undamaged plants (Harris 1974). The compensatory responses to herbivore damage may in some cases more than offset the damage caused. It basically depends on how plants respond to attack by insects or other herbivores.

1.3.1 Plant Defences Against Herbivores

Plants are immobile organisms and have to defend themselves against insects and other herbivores. Most plants in natural ecosystems show little or no obvious damage in spite of the presence of wide variety of phytophagous insects in large numbers. Complete defoliation by phytophagous insects is an exception rather than a rule. It has been estimated that on an average, insects consume only around 10% of all annually produced plant biomass (Barbosa and Schulz 1987). This is primarily due to the fact that plants have evolved a diverse range of structural and biochemical characteristics to protect themselves from herbivores. In contrast, insect pest's damage is usually higher in agroecosystem as many of these characteristics have been lost while breeding plants more palatable to human taste and/or outyielding the traditional plant genotypes. There is a need to study these plant defences to exploit them optimally in commercial agriculture.

1.3.1.1 Structural Defences

1.3.1.1.1 Surface Wax Layer(s)

Surface waxes over the epicuticle protect the plant against desiccation, herbivore feeding and pathogen invasion. Wax layers are variable in thickness and structure, and their amount may reach up to several percent of the dry weight of a plant. Wax crystals often act as structural barriers to insect feeding (Jeffree 1986). Further, the mechano- and chemoreceptors on the insect tarsi and mouth parts receive negative tactile and chemical stimuli from the plant surface covered with a wax layer. For instance, leaf epicuticular wax in Brassicaceae results in non-preference for feeding by the flea beetle, *Phyllotreta cruciferae* (Goeze) in (Bodnaryk 1992).

But wax layer may also have the opposite effect by favouring some insects. In several instances, plants with glossy leaf surfaces (reduced wax layer) have also been shown to be less susceptible to insect pests (Eigenbrode and Espelie 1995). As an indirect effect, wax crystals and wax blooms may also impair the adhesion, mobility and effectiveness of predatory insects resulting in an increase of herbivore populations (Eigenbrode et al. 1999).

1.3.1.1.2 Trichomes

The epidermal surface in plant is usually covered with hair-like structures, which are variable in shape, size, location and function (Werker 2000). The hairs present on the aerial parts of a plant are commonly referred to as trichomes, while the term pubescence refers to the collective trichome cover of a plant surface. The trichomes range in size from a few microns to several centimetres, and the shape varies greatly in different species. The trichomes are of two types: non-glandular and glandular (Payne 1978). Non-glandular trichomes may act as physical barriers against the movements of insects over the plant surface or prevent the herbivores' mouth parts from accessing the feeding tissues of the plant (Ram et al. 2004). Glandular trichomes are specialized to secrete a variety of chemicals (Fahn 2000), which act as important chemical barriers against pests and pathogens (Glas et al. 2012). Hooked trichomes of black bean, *Phaseolus vulgaris* Linnaeus, were found to impale the aphid, *Aphis craccivora* Koch (Johanson 1953), and the leafhopper, *Empoasca fabae* (Harris), leading to wounding and death (Pillemer and Tingey 1978). Interestingly, in some cases, trichome density has been observed to be induced in response to insect feeding. Feeding by the cabbage-white butterfly, *Pieris rapae* (Linnaeus), and the cabbage looper, *Trichoplusia ni* (Hubner), on young black mustard, *Brassica nigra* (Linnaeus) W. D. J. Koch, plants resulted in increased trichome density on newly expanded leaves (Traw and Dawson 2002). Some insect pests have also been reported to have developed morphological or biochemical adaptations to neutralize the effect of trichomes. Trichomes may also have indirect effects on plant resistance by limiting the searching capacity of natural enemies of herbivores. The parasitic wasp, *Encarsia formosa* Gahan, is considerably more efficient in finding its host – whitefly nymphs – on glabrous cultivars than on hairy leaves (van Lenteren et al. 1995).

1.3.1.1.3 Plant Toughness

Coley (1983) observed that leaf toughness was the best predictor of interspecific variation in herbivory rates, in a lowland tropical forest. Plant cell walls strengthened by deposition of macromolecules such as cellulose, lignin, suberin and callose together with sclerenchymatous fibres make a plant resistant to penetration by mouth parts (piercing sucking) and ovipositors (adult females) of insects as well as tearing action of mandibles of chewing insects. In wheat, solid-stemmed cultivars were resistant to stem sawfly, *Cephus cinctus* Norton (Platt and Farstad 1946). In sugarcane, rind hardness was an important factor in reducing internode borer *Diatraea saccharalis* (Fabricius) damage (Martin et al. 1975). Seed damage due to the seed chalcid *Bruchophagus roddi* (Gussakovsky) in alfalfa was less in genotypes with highly lignified pod walls (Springer et al. 1990).

1.3.1.1.4 Plant Architecture

The suitability of a plant to serve as a host for phytophagous insects may vary with plant size and architecture. Plant characteristics such as canopy spacing; stem, leaf and bud shapes and dimensions; and branching angles may affect insect preferences and survival. The increasing size and architectural complexity of plants from monocots through herbs, to bushes and trees, is correlated with an increase in the diversity of the associated insect fauna (Lawton 1983). Indirect effects of plant architecture on herbivores are also mediated through their influence on the natural enemies. In cotton, okra-leaved cultivars suffer less damage by a number of insect pests including bollworms, whitefly and boll weevil as compared to normal-leaved cultivars (Ram et al. 2004). In soybean, cultivars with smaller cotyledons and unifoliate leaves were resistant to the legume seedling fly, *Ophiomyia phaseoli* (Tryon), and these are the parts where the insect lays eggs (Talekar and Tengkano 1993).

1.3.1.2 Biochemical Defences

Plants have evolved a plethora of chemical structures to prevent colonization by insects and other herbivores. While a limited number of chemicals are involved in primary metabolism, many other compounds have been found to repel, deter, kill or prevent insects and other herbivores from utilizing these plants as food sources (Chapman 1974; Harborne 1993; Mithofer and Boland 2012). As phytophagous insects have developed the ability to exploit their hosts, the plants have responded by evolving defensive biochemicals to counteract herbivore attack (Johnson 2011). The chemicals produced by plants, thus, fall into two broad categories: nutrients and allelochemicals.

1.3.1.2.1 Nutrients

The suitability of a plant as a host for one or more insect species is dependent on its ability to supply holistic nutrients for development and multiplication of these insects. From an insect's perspective, the plants usually supply a mixture of nutrients at suboptimal concentrations, which are combined with indigestible structural compounds, such as cellulose and lignin, and a variety of allelochemicals (Schoonhoven et al. 2005). The latter may exert a wide range of behavioural,

physiological and growth-inhibiting effects, some of which may even lead to insect mortality.

Most insects have qualitatively similar nutritional requirements, consisting of carbohydrates, amino acids, fatty acids, sterols and a number of micronutrients. Host plants are often nutritionally suboptimal per se. The main groups of primary plant metabolites – amino acids, carbohydrates and lipids involved in fundamental plant physiological processes – serve as essential nutrients for herbivores. Therefore, changes in primary plant metabolites and nutrients greatly affect the survival and multiplication of phytophagous insects (Berenbaum 1995).

Nitrogen is especially important as insects are unable to exploit inorganic nitrogen, and organic nitrogen content of plants is suboptimal for the insects (Schoonhoven et al. 2005). This may constitute a major barrier to successful exploitation of plants by a majority of insect taxa (orders). Interestingly, the herbivorous taxa include nearly half of the total arthropod fauna in less than one-third of insect orders, indicating that once the nitrogen deficiency barrier is breached, these organisms are able to access an abundant supply of food (Strong et al. 1984).

1.3.1.2.2 Selected Examples of Nutritional Factors in Plant Defence Against Insects

The host plant, which is deficient in one or more essential nutrients required by the insect, may prove insect resistant by causing antibiotic and antixenotic effects on the insect. Such effects could also result from an imbalance of available nutrients (Arora and Dhaliwal 2004).

Cotton Cotton genotypes with inbuilt defence based on nutritional factors have been evolved for insects such as the leafhopper, *Amrasca biguttula* (Ishida); whitefly, *Bemisia tabaci* (Gennadius); stem weevil, *Pempherulus affinis* (Faust); and the thrips complex (Uthamasamy 1996). The whitefly *B. tabaci*-resistant genotypes showed higher contents of K, P and Mg and lower of N and Fe as compared to susceptible ones. But the other parameters like sugars, proteins, Ca and Cu did not show significant relationship with whitefly buildup. In another study, it was reported that total sugar content of cotton cultivars was positively correlated with whitefly incidence during the vegetative phase but negatively correlated with it after flowering of the crop (Rao et al. 1990). In the case of leafhopper, *A. biguttula*, highly susceptible genotype Acala 4–42 had higher amount of reducing sugars (2.55%), proteins (18.49%) and free amino acids (10.15 mg/g) as compared to highly resistant BJR 741 containing 1.63% reducing sugar, 13.45% proteins and 6 mg/g free amino acids (Singh and Agarwal 1988).

Rice The thrips, *Stenchaetothrips biformis* (Bagnall)-resistant rice genotypes possessed significantly less reducing sugars and free amino acids in comparison with the susceptible genotypes (Thayumanavan et al. 1990). The occurrence of asparagine in minute quantities in rice variety 'Mudgo' was considered to be the primary cause of resistance to brown plant hopper, *Nilaparvata lugens* (Stal). Young females of brown plant hopper caged on variety Mudgo had underdeveloped ovaries con-

taining few eggs, while those caged on susceptible varieties had normal ovaries full of eggs (Sogawa and Pathak 1970). The gall midge *Orseolia oryzae* (Wood-Mason)-resistant varieties PTB 18, PTB 21 and Leuang 152 had higher content of free amino acids and less sugar in their shoot apices than susceptible varieties Jaya and IR8. In the case of stem borer, *Scirpophaga incertulas* (Walker), stems of both the resistant (TKM6) and moderately resistant (Ratna) genotypes had less amino acids and sugars than susceptible genotype (IR8) (Vidyachandra et al. 1981).

Legumes The importance of amino acid concentration in the pea plant on susceptibility to aphid, *Acyrthosiphon pisum* (Harris), was revealed by Auclair (1963). He observed that the concentrations of amino acids in the sap of susceptible genotypes were significantly higher than those in the resistant genotypes. It has been reported that high percentage of non-reducing sugars and low percentage of starch in the seeds of chickpea genotype GL 645 might be responsible for the low incidence of the pod borer *H. armigera* in the test cultivar as compared to the infestor (Chhabra et al. 1990).

Low amino acid, protein and sugar contents and high phenol content induced resistance in pigeon pea cultivars against pod borers. Sugar content was high both in seeds (3.64–4.82%) and in the pod coat (3.66–4.92%) of susceptible cultivars (ICPLI, ICPLS7 and UP AS20). In the resistant cultivars, the total sugar content ranged between 2.86 (ICPLS3024) and 3.51% (HS9–2) in the seeds and 2.91 (ICPLS3024) and 3.44% (HS9–2) in the pod coat. The amino acid content was low in the pod coat (1.40–1.52 mg/g) and seed (1.39–1.55 mg/g) of resistant pigeon pea cultivars tested as compared to the susceptible cultivars (1.89–2.57 mg/g in pod coat; 2.04–2.62 mg/g in seed). Highly significant positive correlation observed between amino acid content and incidence of individual borer species supported the possible role of amino acids in offering resistance to the pod borers (Sahoo and Patnaik 2003).

1.3.1.2.3 Allelochemicals

The plant-produced allelochemicals are mainly secondary metabolites which do not play major role in primary metabolic pathways of plants. While the primary metabolic pathways are common in almost all flowering plants, these secondary substances vary widely in different plant species (Schoonhoven et al. 2005). It was Fraenkel (1959) who first postulated that these substances act to deter insects and other herbivores. It has been observed that the plant produce a dazzling variety of secondary metabolites, and more than 200,000 of these have been identified (Dixon and Strack 2003).

The allelochemicals have been functionally classified into two categories: *allomones* which benefit the producing organism, i.e. the host plant, and *kairomones* – which benefit the organism perceiving it, i.e. the phytophagous insect. The involvement of allelochemicals in various types of insect-plant relationships can determine the status of a plant either as a host (presence of kairomone) and non-host (absence of kairomone) or as resistant (presence of allomone) and susceptible (absence of allomone) (Panda and Khush 1995). Allomones are considered a major

Table 1.1 Major groups of phytochemicals utilized in plant defences

Phytochemical group	Example	Typical plant source	Approximate number of compounds known
Terpenoids	(E)-β-Farnesene cucurbitacins	Ubiquitous	>30,000
Steroids	Phytoecdysteroids	Ranunculaceae	~200
Cardenolides	Digoxigenin	Plantaginaceae	~200
Alkaloids	Nicotine	Solanaceae	>12,000
Fatty acid derivatives	(3Z)-Hexenylacetate	Ubiquitous	Not available
Glucosinolates	Sinigrin	Capparales	~150
Cyanogenic glucosides	Dhurrin	Rosaceae, Fabaceae	~60
Phenolics	Simple phenols, coumarins, lignin, tannin	Ubiquitous	>9000
Polypeptides	Trypsin inhibitor	Ubiquitous	Not available
Nonprotein amino acids	γ-Aminobutyric acid	Fabaceae	>200
Silica	SiO_2	Poaceae	1
Latex	Undefined emulsion	Euphorbiaceae	Not available

Modified from Mithofer and Boland (2012)

factor responsible for plant defence against insects, and these have been exploited to increase levels of resistance in several agricultural crops (Green and Hedin 1986). The various groups of secondary plant metabolites implicated in plant defence against insects (Table 1.1) are briefly discussed here (Rosenthal and Berenbaum 1991; Arora and Dhaliwal 2004; Schoonhoven et al. 2005; Iason et al. 2012).

Nonprotein Amino Acids The nonprotein or unusual amino acids are common in a number of unrelated families of higher plants as well as in some lower plants. At least 600 such amino acids have been elucidated from various plants especially legumes. Nonprotein amino acids may afford protection against predators and pathogens due to their structural analogy to the common nutritionally important amino acids. The biological effects on insects are partly due to the fact that the analogue molecule gets misincorporated into protein synthesis of the insect or through inhibition of biosynthetic pathways (Rosenthal 1991; Huang et al. 2011; Yan et al. 2015). Among these, canavanine, azetidine-2-carboxylic acid, 2,4-diaminobutyric acid, mimosine, 3-hydroxyproline, 5-hydroxynorvaline, β-cyanoalanine and pipecolic acid are significant in causing insect growth disruption (Parmar and Walia 2001, Yan et al. 2015).

Terpenoids Terpenoids are the largest and most diverse class of organic compounds found in plants. They exhibit enormous chemical variety and complexity, but all are formed by fusion of five-carbon isopentane units, and most of them are lipophilic substances (Ruzicka 1953). Terpenoids achieve their greatest structural

and functional diversity in the plant kingdom. Nearly 30,000 terpenoids are known in plants, and a majority of them serve as defences against herbivores and pathogens or as attractants for pollinators and fruit-dispersing animals. The terpenoids are constituted of two or more five-carbon units in their structures: monoterpenoids ($2xC_5$), sesquiterpenoids ($3xC_5$) diterpenoids ($4x\ C_5$), triterpenoids ($6xC_5$), tetraterpenoids ($8xC_5$) and polyterpenoids [(C_5) n where n>8] (Gershenzon and Croteau 1991).

Monoterpenoids have been demonstrated to work as toxins and as feeding/oviposition deterrents against a large number of insects. The best known insect toxin among monoterpenoids is the botanical insecticide pyrethrum, found in the flowers and leaves of certain *Chrysanthemum* species. The active ingredient in pyrethrum is a mixture of monoterpene esters collectively known as pyrethroids (Casida 1973).

Cotton and related malvaceous plants possess spherical pigment glands in leaves, flowers and most other parts of the plants. In addition to anthocyanin pigments, these pigment glands contain high concentrations of a variety of mono- and sesquiterpenoids especially gossypol. Gossypol is a phenolic, sesquiterpene dimer with two aldehyde residues. Gossypol is toxic to a variety of herbivorous insects, causing significant decrease in the survival, growth and development of a number of important lepidopterous and coleopterous pests. The toxicity of gossypol to herbivores is supposed to result from its binding to proteins in the gastrointestinal tract, causing a reduction in the rate of protein digestion. The proteins in the gastrointestinal tract may be the ingested dietary proteins or the digestive enzymes produced by the insect (Meisner et al. 1977). The sesquiterpene lactone, *beta*-D-glucopyranosyl ester (TA-G), a major secondary metabolite of the common dandelion, *Taraxacum officinale* G. H. Weber ex Wiggers, protects the plant against its major native root herbivore, the common European cockchafer, *Melolontha melolontha* Linnaeus, by deterring larval feeding (Huber et al. 2016).

Triterpenoids (C_{30}) with six-C_5 isoprene units are the largest of terpenoid compounds. The three major groups of triterpenes which have significant roles in plant-herbivore interactions are the cucurbitacins, limonoids and saponins. Cucurbitacins are a group of about 20 extremely bitter and toxic tetracyclic triterpenes, confined mainly to plants in the Cucurbitaceae family. These compounds serve as toxicants and feeding deterrents against a wide range of phytophagous insects (Tallamy et al. 1997). Some specialist insects feeding on cucurbits are, however, able to metabolize or avoid these toxic compounds and even use cucurbitacins as host recognition cues (Abe and Matsuda 2000).

The limonoids are a large group of highly oxygenated substances with a basic skeleton of 26 carbon atoms. Limonoids are found in three closely related families, the Rutaceae, Meliaceae and Cneoraceae. Limonoids are powerful feeding deterrents against insects. Over 100 triterpenoids have been isolated from the neem *(Azadirachta indica* A. Juss.) seeds, and a number of these are active as insect feeding deterrents and antifeedants. Most important of these is the azadirachtin, which is effective at dosages as low as 50 parts per billion. More than 400 species of insects have been reported to be susceptible to neem preparations at various concentrations. In addition to antifeedant effects, neem is reported to affect the survival,

growth, development, vigour and fecundity of insects (Schumutterer 1995; Dhaliwal and Arora 2001).

Saponins are common constituents of a large number of plant species and consist of a sugar moiety (glycoside) linked to a hydrophobic aglycone, which may be a triterpene or a steroid, both of which originate from the C_{30} precursor, squalene. Triterpenoid saponins have been detected in common legumes such as soybeans, beans, peas, tea, spinach, sugar beet and quinoa. Steroidal saponins are found in oats, capsicum, peppers, aubergine, tomato seed, allium and asparagus (Francis et al. 2002). Saponins exert a strong insecticidal action against several orders and cause increased mortality, lowered food intake, weight reduction, growth retardation and moulting defects (Geyter et al. 2007).

Alkaloids The alkaloids are a heterogeneous class of natural products that occur in all classes of living organisms but are most common in plants. Alkaloids generally include basic substances that contain one or more nitrogen atoms, usually in combination as part of a cyclic system. Most of them are derivatives of common amino acids, such as lysine, tyrosine, tryptophan, histidine and ornithine (Facchini 2001). Alkaloids are found in some 20% of the species of flowering plants. Generally, each alkaloid-bearing species displays its own unique, genetically defined alkaloid pattern. Numerous alkaloids have been reported to be toxic or deterrent to insects. Because of their nitrogenous nature, many alkaloids interfere with the key components of acetylcholine transmission in the nervous system. Nicotine and nornicotine derived from tobacco plant were popular as botanical insecticides before the advent of synthetic organic insecticides (Dhaliwal and Arora 2001). Several groups of structurally unrelated alkaloids such as pyrrolizidines, quinolizidines, indole alkaloids, benzylisoquinolines, steroid alkaloids and methylxanthines are feeding deterrents to many insects and other herbivores at dietary concentrations over 0.1% (Schoonhoven et al. 2005).

Glucosinolates Glucosinolates form a small group of about 100 sulphur- or nitrogen-containing distinctive secondary compounds, which act as precursors of mustard oils. Glucosinolates occur commonly in the order Brassicales, including the commercially important family Brassicaceae. Glucosinolates appear to contribute to effective chemical defences against a majority of non-adapted phytophagous insects (Fahey et al. 2001). In the thale cress *Arabidopsis thaliana* (Linnaeus) Heynhold genome, at least 52 genes are involved in glucosinolate biosynthesis (Arabidopsis Genome initiative 2000, Halkier and Gershenzon 2006). When herbivores attack plant tissues, glucosinolates are hydrolysed by the enzyme myrosinase into several herbivore-deterring metabolites (Hopkins et al. 2009). On the other hand, a small minority of adapted (Brassica-feeding) insects are able to utilize glucosinolates in host seeking and host recognition behaviour. Glucosinolates and their volatile hydrolysis products are also used as cues by natural enemies of Brassica-feeding insects (Louda and Mole 1991).

Insect Hormone Mimics and Antagonists The endocrine system is critical for the development, growth, survival and multiplication of insects. Although many insect hormones are known, two powerful hormones, the juvenile hormone (JH) and the ecdysone or moulting hormone (MH), are recognized to play a major role in these processes. The analogues of these hormones are called juvenoids and ecdysteroids, respectively. It is presumed that plants may have developed juvenoids and ecdysteroids as subtle defences against insect pests. Plant species having high ecdysteroid content (> 1000 ppm) are avoided by insects. Farnesol, sesamin, juvabione, sterculic acid, bakuchiol and thujic acid are some of the important juvenoids isolated from plants and are known to disrupt metamorphosis, moulting and reproduction in insects (Bowers 1991).

Proteinase Inhibitors Protease inhibitors (PIs) constitute an abundant and important group of compounds in plants, which have a defensive function against herbivores, especially insect pests (Dunaevsky et al. 2005). Recent studies using microarrays and proteomic approaches have revealed that the protein-based plant defences play a more important role against herbivores then previously realized (Felton 2005; Zhu-Salzman et al. 2008). Defence-related proteins such as arginases, polyphenol oxidases and peroxidases may have antimicrobial properties; others such as chitinases, cysteine proteases, lectins and leucine amino peptidases may be toxic (Zhu-Salzman et al. 2008). However, the anti-insect action of plant proteins is easily inactivated by proteases. These proteolysis-susceptible proteins can be protected with PIs (Mithofer and Boland 2012).

The PIs inhibit the activities of various enzymes in insects especially insect peptidases including serine, cysteine and aspartate proteinases and metallo-carboxypeptidases, which are involved in insect growth and development. The PIs also reduce the digestive ability of the insect pests, thus leading to the shortage of important food constituents such as amino acids resulting in slow development and/or starvation. A large number of PIs have been reported in plants (De Leo et al. 2002), which are effective against many lepidopteran and hemipteran insect pests (War and Sharma 2014). For instance, in tomato plants, PIs were positively tested for their trypsin- and *H. armigera* gut proteinase-inhibitory activity in different parts of the plant (Damle et al. 2005).

Lectins Lectins or phytohaemagglutinins are proteins with a capacity to reversibly bind to the carbohydrate moieties of complex carbohydrates without altering the covalent structure of any of the recognized glycosyl legends. Lectins are distributed universally throughout the plant kingdom, where they constitute 6–11% of the total plant proteins. The cotyledons of the seeds of legumes are especially rich in lectins. Lectins are associated with the defence of plants against insects and phytopathogens (Liener 1991). *Arisaema helleborifolium* Schott lectin exhibited anti-insect activity towards the second instar larvae of melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Kaur et al. 2006).

Phenolics Phenolics are aromatic compounds with one or more hydroxyl groups and are ubiquitous in plants (Harborne 1994). Examples of relatively simple phenolics include hydroxybenzoic acids like vanillic acid, the hydroxycinnamic acids like caffeic acid and the coumarins (Schoonhoven et al. 2005). Coumarins possess a 5,6-benz-2-pyrone skeleton and may be variously hydroxylated, alkylated, alkoxylated or acylated. Coumarins can deter feeding as well as interfere with development of insects. The simple coumarin, bergamottin, is ovicidal to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), while mammein is toxic to the mustard beetles. Coumarins appear to act as kairomones for certain insects that are specialized for feeding on coumarin-containing plants (Berenbaum 1991b).

Among the phenolics, flavonoids are found in nearly all higher plants, and most plants show their own distinctive flavonoid profile. The flavonoids share a basic C_6-C_3-C_6 structure, which is linked to a sugar moiety to form a water soluble glycoside. Common examples of flavonoids isolated from plants are catechin, botanical insecticide rotenone and phaseolin, all of which act as feeding deterrents against insects (Schoonhoven et al. 2005).

Tannins are polyphenolic compounds commonly found in higher plants. The phenolic hydroxyl groups of tannins bind to almost all soluble proteins, producing insoluble copolymers. Proteins bound to tannins are indigestible and thus decrease the nutritional value of plant tissues (Schoonhoven et al. 2005).

Latex Latex is present in specialized cells called laticifers and consists of chemically undefined milky suspensions or emulsions of particles in an aqueous fluid (Agrawal and Konno 2009). Laticifers have a defensive function. Small insects may be physically trapped in latex or their mouthparts may get glued together, and chemical constituents in latex including proteins and toxins affect insect development (Dussourd 1995). Wounding of laticifers by insects results in leakage at wound site (Mithofer and Boland 2012). In the milkweed, *Hoodia gordonii* (Masson) Sweet ex Decne, both larval feeding and adult oviposition by *T. ni* was deterred when latex was added to artificial diet or painted on the leaves of the host plant (Chow et al. 2005).

1.3.1.2.4 Selected Examples of Allelochemicals in Plant Defence Against Insects

Maize Maize, the world's most productive grain crop, is attacked by a diverse range of insect pests. Well-studied anti-herbivore defences in maize include small molecules such as benzoxazinoids (Frey et al. 2009), chlorogenic acid (Cortes-Cruz et al. 2003) and maysin (Rector et al. 2003) in addition to defence-related proteins (Chuang et al. 2014). Xie et al. (1992) analysed several maize lines resistant to western corn rootworm, *Diabrotica virgifera* Le Conte, for hydroxamic acid levels. All the root extracts were found to contain four major hydroxamic acids: 2,4-dihydroxy-7methoxy-l,4-benzoxazin-3-(4H)-one (DIMBOA), 2,4-dihydroxy-7,8dimethoxy-l,4-benzoxazin-3(4H)-one (DIM2BOA), 2-hydroxy,7-methoxy,1,4-benzoxazin-3(4H)-one (HMBOA) and 6-methoxy-benzoxazolinone (MBOA). These chemicals adversely affected the survival development, weight and head