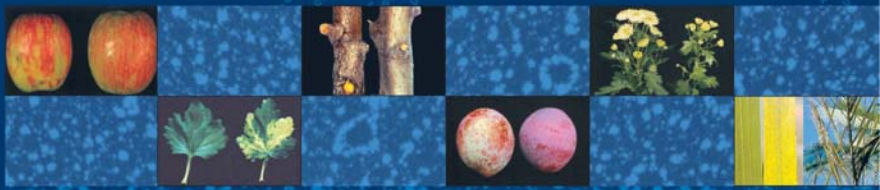
VIROIDS



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VIROIDS

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VIROIDS

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PREFACE

In the early 1950s, James Watson and Francis Crick at the Cavendish Laboratory, Cambridge University, revealed the double helical structure of cellular DNA. At about the same time, Heinz Fraenkel-Conrat and Robley Williams at the Virus Laboratory, University of California, Berkeley demonstrated that the RNA, not the coat protein, of *Tobacco mosaic virus* is the genetic and hereditary component of the virus. These remarkable discoveries and other similar ones in the 1950s marked the birth of the modern era of molecular biology and molecular virology.

Potato spindle tuber disease was identified in North America in the early 1920s. Its effects were the appearance of stunted potato plants and a harvest of spindly pointed tubers, which became a serious problem for potato certification programs. Despite the seriousness of the disease, nothing was known about the causal agent. After eliminating all the other possibilities, plant pathologists concluded that the disease might be caused by a virus. In the early 1960s a systematic study of this disease began. In 1962, the causal agent of potato spindle tuber disease was mechanically transmitted to tomato seedlings, a non-potato indicator plant species for the disease. This was the key element in enhancing research on the etiological agent of the disease because the time span for testing for the infectious agent was greatly reduced from two years in potato to produce spindled tubers to two weeks in tomato to show stunting and epinasty symptoms. By the late 1960s, research had shown that the agent causing spindle tuber disease was evidently not a conventional 'virus', but something entirely new.

Between 1966 and 1968, it was recognized that the causal agent of potato spindle tuber disease, as well as that of citrus exocortis disease, was not a conventional viral nucleoprotein but was most likely a small RNA without coat protein. By 1971, evidence had been presented by two independent laboratories, one in the US and the other in Canada, that a low-molecular weight RNA was the causal agent of potato spindle tuber disease. In 1972, similar findings were reported in the US by a third laboratory for the causal agent of citrus exocortis disease. For both potato spindle tuber and citrus exocortis diseases, the causal RNA agent existed in an unencapsidated form in plants, unlike conventional viruses, and replicated without the help of a virus, thus differing from viral satellite RNAs. Moreover, no message function could be demonstrated. For such an unconventional entity, various terms such as 'viroid' (because it is like a virus), 'metavirus', and 'pathogene' were proposed. The term 'viroid' was eventually accepted. In order to differentiate the abbreviation of viroid from that of virus, for viroids a lower case 'd' is added to the V designation of viruses. Thus *Potato spindle tuber viroid* becomes PSTVd and *Citrus exocortis viroid* becomes CEVd.

Cadang-cadang disease, which causes premature decline and death of coconut palm trees, was first recognized early in the twentieth century in the Philippines. It was not until 1975 that it was determined in Australia that the disease was caused by a viroid, *Coconut cadang-cadang viroid*. It was the first viroid of a monocotyledonous plant species.

Since the mid-1970s viroid research has emphasized conventional aspects such as viroid characterization, viroid structure and replication. However, it has also begun to explore the investigation of viroids as possible causal agents for diseases of uncertain etiology, as well as more practical aspects such as the development of rapid and accurate diagnostic tests to keep viroid diseases out of horticultural, ornamental, and field crops.

Since the demonstrations that potato spindle disease of potato, exocortis disease of citrus, and cadang-cadang disease of coconut are caused by viroids, many plant diseases of once uncertain etiology were identified to have been caused by viroids. Currently, there are approximately 30 known viroid species. They belong in two families, *Pospiviroidae* with five genera and *Avsunviroidae* with two genera. Some of the viroid diseases cause significant damage to the host crop, others are latent in their primary host, but can do considerable damage to other susceptible crops located near the infected latent host species. Such latent hosts may be significant in plant quarantine and germplasm introductions, both in determining the epidemiology of viroid diseases and in formulating control strategies.

In the 10-year period of 1977–1987 three books on viroids were published. They mainly covered advances in viroid research during the first and second decades after the initial viroid discovery. The purpose of the present volume is to serve as an exhaustive reference work about viroids. The editors therefore considered it appropriate that a book presenting such a comprehensive coverage of viroids, in the absence of an overview of the topic for 16

years, would bear the all-embracing title *Viroids*. The book is thus intended to provide a watershed for the next phase of viroid research, which is urgently needed to shed more light on viroid origin, structure and function, pathogenicity, epidemiology, and control. The contributing authors of this volume are an international group of scientists who have substantial experience working with viroids and viroid diseases.

Viroids presents indispensable, comprehensive and up-to-date information pertinent to viroids, viroid diseases, and their control. It provides a single source of information on the economic impact of viroid diseases, properties of viroids, methods for viroid detection and control, diseases of viroids in different plant species, mapping of the geographical distribution and epidemiology of viroids, diseases of possible viroid etiology, and considerations for future applications of viroids. This book also covers plant quarantine and certification programs for viroid diseases. This information will help anyone concerned with the safe movement of plant material across international boundaries or within a single country.

For the benefit of readers, chapters have been grouped into eight parts. A number of chapters focus on the current state of knowledge of the molecular characteristics of viroids, biology, localization and movement, replication, pathogenesis, viroids and gene silencing, classification, viroid-like satellite RNAs; detection of viroids using bioamplification hosts, biological indexing, polyacrylamide gel electrophoresis, molecular hybridization, polymerase chain reaction; mapping of geographical distribution and epidemiology of viroids in North America, Australasia, China, Japan, Europe, the Middle East, Africa, South America, and at the global level. Control of viroids includes quarantine of imported germplasm, availability of viroid-tested propagation materials, thermotherapy, tissue culture, and other conventional strategies as well as biotechnological control approaches. Special topics such as ribozyme reactions of viroids and economic advantages of viroid infection are also included. Chapters that summarize the current state of knowledge concerning viroid diseases of the crop in question and aspects of the natural history of viroids in horticulture are also presented. Among the crops covered are potato, tomato, tobacco, cucumber, pome fruits, stone fruits, avocado, citrus, grapevines, hop, chrysanthemum, coleus, columnea, and coconut palm.

The main aim of the editors has been to produce a cohesive, comprehensive, and up-to-date volume that can be used by students, researchers, extension agents, and regulators. It also may be of great value to science managers, policy makers, and industries in formulating policies and products to obtain viroid-free plants and control viroid diseases.

On behalf of the editors, I would like to thank all contributors, whom I got to know personally over the years, for their willing participation in this project, their patience, and understanding. I would also like to express my gratitude to Mrs Marie Tousignant for her valuable expertise in preparing this book for publication.

A. Hadidi

Part I

Introduction

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CHAPTER 1

ECONOMIC IMPACT OF VIROID DISEASES

J.W. Randles

Viroids are pathogens of food, industrial and ornamental plants. An evaluation of their economic impact faces the same difficulties as encountered for other obligate intracellular biotrophs, such as viruses, phytoplasmas and fastidious bacteria. Viroid diseases are inconspicuous compared with diseases caused by fungi, bacteria and nematodes, and losses identified in comparative trials on their effects do not necessarily translate to global estimates of loss. Their effects also extend beyond direct effects on yield and quality. The range of direct and indirect damage associated with plant virus infection (Waterworth and Hadidi 1998) largely applies also to viroids.

This chapter attempts to consider viroids as one of the many factors that affect world agriculture. It therefore commences with an outline of the history of agricultural development, world food needs and the availability of land. It discusses the role of crop protection. The deployment of new technologies in crop protection and their role in discovering viroids is a precursor to discussing the economic impact of viroids by referring to specific case studies. Crop damage caused by viroids, potential benefits of viroids, and their mode of spread are considered before considering the need for, and the design of appropriate control measures.

FORECAST OF AGRICULTURE, FOOD AND LAND REQUIREMENTS UNTIL 2150

The epoch of science and industrialization is considered to be the 300-year period from 1850 to 2150, in which the world population will show a logistic increase (Weber 1994). Economic divisions between countries with rural and industrial economies can be expected to remain for most of this era. Patterns of population growth for developing countries will be logistic, with an associated demand for greater quantities of food. In contrast, industrialized countries will show a minor increase in population and an associated demand for improved quality of food and lifestyle. It is estimated that the current world population will double to a peak of about 12×10^9 in the next 120–150 years, a limit imposed by the consumption of more resources and pollution by industrial and agricultural wastes rather than an inability to expand food production. The individual mean energy requirement of food is about 3000 cal per day, and the projected doubling of the world population, together with an increased demand for quality, will require research and development of new products and processes, as well as world peace and international support for poorer countries (Weber 1994). Up to 3×10^9 ha of land could be cultivated

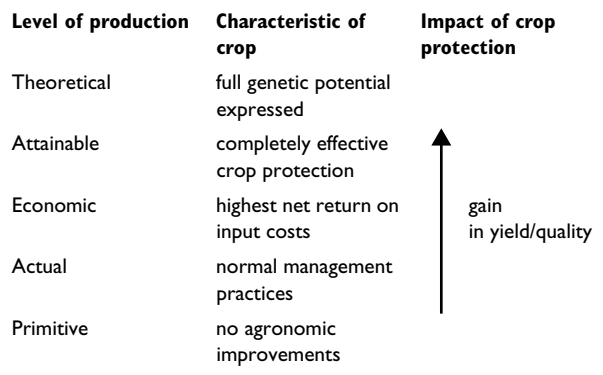

Figure 1.1 The impact of crop protection on crop production. The 'actual' level achievable at a site where normal practices are used is compared with the level 'attainable' when all pests are absent. The impact of crop protection falls within the range indicated by the arrow (based on Oerke *et al.* 1994).

worldwide and, with improvements in agriculture, should be adequate for the expected limit of world population. However, the cost will be further clearance of natural vegetation with associated deleterious effects on ecosystems and loss of biodiversity.

Land-saving advances in technology contributing to improvements in food production will continue to be the use of chemical fertilizers, plant breeding and crop protection. Of these, crop protection is the most complex because of the range of crops, range of pests, pathogens and weeds, and the different life cycles of each of these. Also, the need for crop protection increases with increases in crop yield because of the higher plant density, shortened intercrop periods, monocultures, and the increased use of fertilizers and irrigation. An essential component of crop protection in the future will be the generation of information through research and the use of this information for control of crop losses.

Crop losses and crop protection

Theoretical levels of crop production are summarized in Figure 1.1. Crop loss can be defined as the difference between actual yield under normal optimum agronomic conditions and the yield attainable for the same crop with completely effective crop protection. The effect of crop protection is a gain in yield or quality. Economic yield is the level at which incremental costs of crop protection reach but do not exceed the incremental increase in value of the crop. Ideally, estimates of loss would be a prerequisite for the rational development of plant protection programs, but crop loss assessment is complex and inexact (Oerke 1994). An estimated global figure for preharvest crop loss from all sources is considered to be about 40%, while postharvest losses are estimated to be in the range of 10–50%. Thus, more than 50% of produce grown is lost before reaching the consumer.

In plant pathology, research has provided better diagnostic methods, more accurate information on epidemiology, disease forecasting, cultural control measures and novel forms of resistance. Integrated crop management combines these with other strategies as a basis for sustainable agriculture, including concern for natural resources and represents a logical way forward between the extremes of ultra-intensive agrosystems and low output organic farming (Dehne and Schonbeck 1994). Because of high costs and the need for research infrastructure, these measures are first adopted by developed countries. Developing countries may only invest in selected techniques for use in crops destined for export to countries which impose strict tolerances on food quality or plant health.

The deployment of new technologies in plant pathology

The range of techniques available does not provide an adequate solution to all disease losses. Where therapeutic means of control are not available, as for the intracellular biotrophic pathogens, indirect control measures are needed, but they can only be applied if the disease cycle is known. Such solutions are often temporary and require continued intellectual input to remain relevant to the requirement for sustainability. Biotechnology and genetic engineering are the most recent contributors to new control strategies. Direct incorporation of resistance genes against many plant viruses by transformation of plants with pathogen-derived nucleotide sequences is now well tested and the phenomenon of gene silencing is now under intensive study (Hull 2002). Moreover, the development of highly sensitive and specific nucleic acid based diagnostic tests promises to revolutionize quarantine practice and pathogen-testing schemes for exclusion of infected plant material. The cost effectiveness of this technology is now widely recognized and it only remains for it to be deployed where it is likely to be most effective.

Research on viroids over the last 30 years has paralleled and contributed to the development of biotechnology. The discovery of the first viroid, *Potato spindle tuber viroid* (PSTVd), exemplifies the ground-breaking consequences of identifying a new paradigm in plant biochemistry and pathology. The positive economic impact of the original discoveries that previously enigmatic diseases were due to viroids has been greatly multiplied not only by the ability of workers in quarantine and pathogen-testing schemes to identify, evaluate and control viroids, but by their ongoing contribution to understanding cellular processes in molecular biology and particularly the puzzle of how they produce disease. This positive economic impact is highly significant but impossible to quantify because many of the long-term consequences are not obvious.

Losses due to viroids

Viroids vary in the hosts they infect, the type and severity of disease that they cause, their mode of spread and their epidemiol-

ogy. They also vary in pathogenicity, time to induce disease, interactions with other pathogens and response to the environment. Control measures may be available for some, while the lack of knowledge of the epidemiology may prevent the formulation of reliable control strategies for others. Many of the known viroids were discovered because they induced serious damage to their host crops. Following their characterization, attempts were made to monitor their incidence and effects. This was intended to lead to quantification of the dynamics of incidence, spread and distribution, followed by the prediction of outbreaks and the deployment of control treatments.

As each viroid has a unique disease cycle, each disease cycle needs to be defined so that a specific system of assessment, loss prediction and control can be established. The measurement of losses due to viroids has two aspects. The first is the severity of the disease induced by infection with the viroid and its variants. The second is the prevalence of the viroid and its ability to spread and produce an epidemic. Direct losses can be measured either as a depression in yield or as a cash loss. These losses will vary with the viroid, the crop, time and the environment. The types of loss that have been reported from viroid infection include losses of whole plants, damage to parts of plants, damage to plant products, damage to subsequent generations, and the effects of coinfection with other endoparasitic pathogens.

Whole plant losses

The effect of lethal viroid diseases of coconut palm such as cadang-cadang and tinangaja, as well as those leading to the removal of unproductive annual or perennial plants, can be evaluated using a descriptive formula:

Loss = value of plant
+ cost of replacement
+ time lost from interrupted production
+ cost of diagnosis

This implies that where viroid control is achieved by eradication and replanting, the effect on loss will be the same as natural death of the plant. This situation allows individual losses to be calculated with reasonable accuracy.

Damage to parts of plants

Specific tissues and organs of plants may be directly affected, or a general reduction in growth rate may cause stunting and reduced yield. For example, foliage may be distorted, chlorotic or damaged through increased susceptibility to sun or wind. Plant structure may be affected by reduced growth, lesions on the stem or reduced translocation of water and nutrients. Reproductive structures may be affected. For example, flower production may cease, or flowers may show necrosis, reduced size or change in pigmentation. Fruit may be altered in size, shape, quality, color or markings. Seed may be small or shrivelled, with changed composition or viability.

Meristems, at both apex and root tips, may show reduced cell division, altered patterns of differentiation leading to hypoplasia or hyperplasia in plant tissues. Any reduction in cell division will be a precursor of reduced growth rate in both roots and tops of plants. This may be reflected in shortened internodes or loss of apical dominance in dicotyledonous plants, or 'pencilling' of the trunks of monocotyledons such as palms. These reduced outputs by the plant occur despite normal inputs of labor, nutrients and water.

Damage to plant products

If the economic product of the plant is a tuber, nut or fruit, downgrading of quality, predisposition to damage at harvesting or in storage, and reduced yield at harvest, may be components of loss. Seed viability, strength of the plant's framework and of timber harvested from the plant may be reduced by viroid infection and incur a cost. Variation in growth rates of infected compared with viroid-free plants may incur labor costs in plantation management, as for hops with reduced extension of bines when infected with *Hop stunt viroid* (HSVd).

Effects on subsequent generations

If a viroid is transferred to the next generation of the host species, in true seed or vegetative propagules, the rate of transmission and the cost of an epidemic resulting from secondary spread from these primary sources of infection will determine the extent of future losses.

Mixed infections

A range of consequences may arise from mixed infection either with different strains of viroids, with different viroids or with viroids and viruses (Garnsey and Randles 1987).

Cross-protection occurs between mild and severe isolates of several viroids. It has not been used as a control measure for viroids, but has been used to identify mild strains of PSTVd in biological indexing programs. Molecular analysis has indicated that for viroid-infected perennial crops such as grapevine, stonefruit and citrus, a most important consequence of mixed infection is the appearance of recombinant 'new' viroids, with the potential for genetic properties to be derived from several co-infecting viroids. Synergism has been reported in potato between PSTVd and *Potato virus Y* (Singh 1982). The report (Francki *et al.* 1986) that PSTVd could be encapsidated in *Velvet tobacco mottle virus*, which is mirid transmitted in nature, indicated that co-infection may have major consequences for viroid epidemiology by providing an unexpected mode of spread. Salazar *et al.* (1995) reported very high rates of transmission of PSTVd by *Myzus persicae* when source plants were doubly infected with *Potato leafroll virus* (PLRV) and PSTVd. The epidemiological consequences of this report are very important because of the widespread distribution of PLRV.