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Control of Plant Pathogens with Viruses and Related Agents [and Discussion]

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Control of plant pathogens with viruses and related agents

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Cytoplasmically transmissible agents causing diseases of plant pathogenic fungi characterized by reductions in pathogenicity, ability to form sexual and asexual spores, spore viability and growth rate, are often associated with the presence of one or more specific segments of virus-like double-stranded RNA (dsRNA). In Italy, hypovirulent dsRNA-containing strains of the chestnut blight fungus, *Endothia* (*Cryphonectria*) *parasitica*, have become predominant in many areas where blight is no longer a serious problem. dsRNA-containing strains of other pathogens, with various degrees of debilitation, survive in natural populations but have not become predominant or resulted in any great reduction in disease. Examples include the Dutch elm disease fungus, *Ophiostoma* (*Ceratocystis*) *ulmi*, and the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*. Successful biological control of such pathogens could probably be achieved, however, if methods could be developed to suppress the loss of dsRNA that occurs during the sexual and other stages of their life cycles, and to suppress the vegetative incompatibility reactions that reduce the cytoplasmic transmission of dsRNA. Systemic infection with attenuated strains of plant viruses can protect plants from later infection by virulent strains of the same or closely related viruses. Despite some notable successes, e.g. control of citrus tristeza and tomato mosaic viruses, such 'cross-protection' has not been widely applied because of the cost and difficulty of application, and caution about the widespread distribution of infectious agents in the environment. These problems could be overcome if cross-protection could be achieved by the expression of a single viral gene rather than infection with intact virus, and consideration of possible mechanisms of cross protection suggests novel ways of producing virus-resistant plants.

1. Introduction

There are a number of ways in which viruses might be used to control plant pathogens: (1) viruses may kill or reduce the pathogenicity of bacterial and fungal pathogens of plants; (2) viruses may kill invertebrate vectors of viruses of higher plants and thus prevent their spread; (3) mild strains causing little or no disease may protect plant hosts from later infection by more severe strains. Vidaver (1976) considered that bacteriocins were more promising and less risky than bacteriophages for control of bacterial plant pathogens, whereas the current status of insect viruses in biocontrol systems (Payne, this symposium) makes it unlikely that they will replace chemical pesticides in controlling insect vectors of plant viruses. In contrast, the recovery of blighted chestnuts in many areas of Italy associated with the spread of virus-like nucleic acids through the population of the pathogen *Endothia* (*Cryphonectria*) *parasitica* is one of the few known examples of natural biological control of a plant disease. Likewise, the protection of citrus trees in Brazil by pre-infection with mild strains of citrus tristeza virus is a spectacular example of biological control of a plant virus by the intervention of man. Reasons why these biocontrol systems have been successful, and the possibilities of applying similar

principles to other systems, will be discussed. Examples of novel ways of producing virus-resistant plants arising from consideration of the mechanisms of biological control will be presented.

2. Viruses of plant pathogenic fungi and their potential as biological control agents

(*a*) *General properties of fungal viruses*

(i) *Occurrence*

Viral infections of fungi are common and have been found in all the major fungal subdivisions. Within some species a large proportion of individuals may be infected; for example, Stanway (1985) detected virus infection in 126 out of 157 field isolates of the wheat take-all fungus. *Gaeumannomyces graminis* var. *tritici*. Several morphological types of particle have been detected in different fungi, but isometric viruses with genomes of double-stranded RNA (dsRNA) are most frequent (Buck 1986*a*). In a few fungi, dsRNA is not enclosed in isometric particles. In *E. parasitica* the dsRNA is enclosed in lipid-rich vesicles (Hansen *et al.* 1985). The structural properties of the dsRNA (Tartaglia *et al.* 1986; Hiremath *et al.* 1986) and its association with membrane structures that contain RNA-dependent RNA polymerase activity (Hansen *et al.* 1985) suggest a viral origin, although it has also been proposed that the dsRNA may be the replicative form of a single-stranded RNA virus (Tartaglia *et al.* 1986). In *Ophiostoma* (*Ceratocystis*) *ulmi* dsRNA is associated with the mitochondria (Rogers 1987; Rogers *et al.* 1988*a*).

(ii) *Transmission*

Viruses of fungi differ from those of other hosts in that they generally do not lyse their host cells and are apparently transmitted only by intracellular routes (Buck 1986*a*). Within a fungal strain, virus particles are carried forward in the net flow of protoplasm towards the growing hyphal tip so that a fungus maintained in the mycelial state may remain infected indefinitely. Transmission into asexual spores is usually very efficient, although partial or complete loss of viral genomes has been observed at low frequency during conidiogenesis in some fungi.

Transmission between fungal strains can occur after fusion between somatic or sexual cells of the same species. Transmission by hyphal anastomosis can be limited further by vegetative incompatibility reactions when one or more vegetative compatibility (v-c) genes of the individuals are different. A fungus may have as many as seven v-c genes giving 128 possible v-c types, assuming two alleles of each gene. Transmission is usually very efficient when all v-c genes are the same, becomes increasingly less efficient with increasing number of v-c gene differences, with modulating effects caused by differing 'strengths' of individual v-c genes and different host genetic backgrounds (Anagnostakis 1983, 1984; Brasier 1984, 1986*a*). Sexual transmission of viruses will depend (for heterothallic organisms) on compatibility of mating-type genes. In the Basidiomycetes, transmission of viruses into sexual spores appears to be efficient, although incompatibility between dsRNA segments of different strains can lead to exclusion of some segments, e.g. in the corn smut fungus, *Ustilago maydis* (Wigderson & Koltin 1982). In a number of Ascomycetes host exclusion mechanisms result in the absence in ascospore progeny of all or most of the dsRNA segments of the parents, e.g. in *G. graminis* var.

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tritici (McFadden *et al.* 1983), *O. ulmi* (Rogers *et al.* 1986*a*) and *Helminthosporium maydis* (Bozarth 1977). Overall, the ability of a virus to spread through a fungal population will depend on the heterogeneity of populations with respect to v-c and mating-type genes, possible host- or dsRNA-mediated exclusion reactions and the relative importance of the sexual stage in the biology of the fungus.

(iii) *Effect on fungal phenotype*

Many fungi tolerate viral infection without any apparent adverse effect. However, viral infection can alter a fungal phenotype, not as a general result of the presence of viral particles or dsRNA, but as a consequence of the expression of specific coding sequences within one or more dsRNA segments. For example, killer strains of *U. maydis* secrete protein toxins that kill sensitive strains of the same or related species. Many isolates of *U. maydis* contain multiple segments of dsRNA (Day 1981), but only those containing the specific dsRNA segments encoding protein toxins and immunity from them are killers (Koltin 1986; Shelbourn *et al.* 1987) and these are only a small proportion of natural populations (Day & Dodds 1979). Ability to produce killer toxin does not appear to alter the pathogenicity of *U. maydis* strains. However, Koltin & Day (1975) suggested that if the toxin-encoding sequences could be introduced into the genome of cereals and expressed by the plants, smut-resistant plants could be produced.

In some instances various degrees of debilitation or disease of the fungus, such as abnormal morphology, slow growth, reduced sexual and asexual sporulation, and a marked decrease in pathogenicity (often termed hypovirulence) have been shown to be associated with specific dsRNA segments. Examples include diseased or hypovirulent strains of *E. parasitica* (Van Alfen 1986), *O. ulmi* (Rogers *et al.* 1986*a*), *Helminthosporium victoriae* (Ghabrial 1986) and *Rhizoctonia solani* (Castanho & Butler 1978*a*). The role of specific dsRNA segments is inferred from observations that their transmission by hyphal anastomosis results in conversion of healthy recipients to the diseased phenotype, whereas loss of segments from diseased isolates results in recovery from the disease; proof by direct infection of healthy isolates with viral particles or dsRNA has not yet been achieved and hence the possibility that these phenotypes might be caused by defective mitochondrial DNA or DNA plasmids (Bockelmann *et al.* 1986) cannot be excluded.

(*b*) *Strategies for biological control*

Weakly pathogenic or nonpathogenic variants of a plant pathogen or related species may have the ability to protect plants from attack by the pathogen, e.g. control of *G. graminis* var. *tritici* by *Phialophora* spp. (Wong 1981). Protection may result from direct competition by prior occupation of infection sites on the host and from induction of host resistance. In weakly pathogenic organisms having multiple differences from the pathogen at the nuclear rather than the cytoplasmic level, the phenotype is not cytoplasmically transmitted. In contrast, when hypovirulence results from cytoplasmic genetic elements (H-factors), such as dsRNA, the hypovirulent strains have the potential to control a pathogen even after the latter has infected its host. Such curative properties are the result of transmission of H-factors from the weakly pathogenic (hypovirulent) to the strongly pathogenic (virulent) strains, making the latter hypovirulent.

The strategy for biological control by using isolates with cytoplasmically determined

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hypovirulence will depend on several variables, such as the nature of the host–pathogen interaction, the biological cycle of the pathogen, the properties of the virus-infected pathogen, the transmissibility of the virus and whether the host is annual or perennial. To illustrate how such variables may affect the strategy, two tree pathogens with different biological cycles, namely *E. parasitica* and *O. ulmi*, will be compared. Possibilities for control of annual crops with hypovirulent strains will then be assessed and ways of resolving current problems will be considered.

(*c*) *Recovery of blighted chestnuts in Italy: an example of natural biological control*

The epidemic of chestnut blight in North America which started around the beginning of the twentieth century has eliminated the American chestnut, *Castanea dentata*, as an important economic tree (reviewed by Elliston (1982), Anagnostakis (1982) and Van Alfen (1982, 1986)). This devastating canker disease is caused by the fungus *E. parasitica*, which is spread by both sexual spores (ascospores), adapted for transmission by wind, and asexual spores (conidia) adapted for transmission by rain and possibly by insects, small mammals and birds. Infection is initiated at wounds in the bark, and trees are killed by mycelial fans that rapidly invade bark tissue encircling and girdling the branch or trunk, penetrate the cambium and block the vascular system. The roots of killed trees produce sprouts, but these generally only survive for up to four or five years before being killed by *E. parasitica*.

Chestnut blight was first found in Italy in 1938 and by 1950 was widely distributed in the northern and southern chestnut growing regions of the country. However, soon afterwards in a chestnut coppice near Genoa, once severely damaged by *E. parasitica*, it was noticed that although a large proportion of shoots were infected, only a few showed characteristic symptoms. On most shoots cankers were healing and the fungus was restricted to the outer layer of bark (Biraghi 1953). Isolates from the bark of such trees differed from normal *E. parasitica* in morphology and degree of pigmentation, produced fewer conidia more slowly, were nonpathogenic or only weakly pathogenic to *Castanea sativa*, and when inoculated into cankers cured existing blight (Grente 1965; Grente & Sauret 1969). A small proportion of conidia of such hypovirulent (H) strains reverted to normal virulent (V) strains, confirming their identity as *E. parasitica* and suggesting that hypovirulence is caused by a cytoplasmic genetic element (H-factor). This was proved by transmitting H-factors by hyphal anastomosis (Berthelay-Sauret 1973; Van Alfen *et al.* 1975), and correlative evidence strongly suggests that H-factors are dsRNA molecules (Day *et al.* 1977; Fulbright 1984; Elliston 1985). The spread of H strains in Italy led to a steady decline in the incidence of chestnut blight so that by 1978 it was no longer a serious problem (Mittempergher 1978; Turchetti 1978, 1979).

The current dominance of hypovirulent strains that has led to the re-establishment of the chestnut in Italy stems from a combination of several factors.

1. H strains parasitize chestnut trees but produce only superficial cankers and do not cause significant disease. There appears to be little difference in initial responses of chestnuts that are wounded and immediately inoculated with H or V strains (Hebard *et al.* 1984). In both cases, lesions or wounds are delimited initially by a zone of lignified tissue and formation of wound periderm begins at the deepest part of the wound and progresses outwards to the bark surface. Fully formed wound periderm appears to be completely resistant to mycelial fans of V or H strains. However, mycelial fans of V strains develop quickly, penetrate the lignified zone and developing wound periderm, halt wound periderm formation by killing cells in front of the

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advancing hyphae and eventually colonize the vascular cambium. In contrast, mycelial fans of H strains develop more slowly and, although they can penetrate lignified zones and developing periderm, are blocked by fully formed periderm before they reach the vascular cambium. The host response therefore develops rapidly enough to control the slow-growing H strains, but not the faster-growing V strains. This explanation is consistent with the observation that even H strains can infect newly made graft wounds and kill scions of hybrid chestnuts that can only be propagated vegetatively (Turchetti 1979), presumably because they colonize exposed vascular cambium before it is protected by callus tissue.

2. Although H strains are less vigorous than V strains in that they develop more slowly on chestnut trees and produce fewer conidia, they are not too debilitated to survive in nature.

3. H strains compete successfully with compatible V strains. This is due to transmission of the cytoplasmic determinants for hypovirulence from H to V strains resulting in 'hypovirulence conversion', and not to direct competition. In fact, when H-factor transmission is blocked by vegetative incompatibility, H strains compete poorly with V strains and do not arrest canker development (Anagnostakis 1982). In Italy, conversion to hypovirulence seems to have been facilitated by strains belonging to only one or a few v-c groups in the regions where H strains have become predominant.

4. H strains do not readily revert to virulence because most conidia of H strains are hypovirulent (Van Alfen *et al.* 1978), and no European H strains are known to produce ascospores (Elliston 1982). By depressing the sexual stage of their hosts, H factors have promoted their own survival because dsRNA is normally lost during ascospore formation.

5. H strains can be transmitted from tree to tree. Their efficient spread is essential not only for survival, but also for control of V strains. Prior infection with H strains does not protect trees from infection with V strains in other locations on the tree; to be effective in control, H strains need the capacity to be transmitted to newly formed cankers caused by V strains.

In Italy all the above factors favour the establishment of equilibria not only between hypovirulent strains and the European chestnut but also between H-factors (which can be regarded as hyperparasites) and *E. parasitica*. As a result, Italian forest chestnuts are still being used as a source of timber.

Limited success in controlling blight in chestnut orchards in France and Switzerland has been obtained by treating cankers with mixtures of H strains in different v-c groups. In areas where V strains belong to one or a few v-c groups there is evidence for spread of H strains and their curative properties to other trees in the locality (Grente 1981; Bazzigher *et al.* 1981).

In North America, H strains have been obtained from naturally recovering chestnut trees in Michigan, Pennsylvania, Tennesse and Virginia (Anagnostakis 1982) with evidence for their spread in Michigan (Fulbright *et al.* 1983). Both European and American H strains are heterogeneous, varying in degree of pigmentation, morphology, sporulation and reduction of pathogenicity. This variation is probably due, at least in part, to variation of the dsRNA segments. Recent evidence suggests that a single molecule of dsRNA of about 9000 nucleotides may be sufficient to confer the hypovirulent phenotype (Hiremath *et al.* 1986; Tartaglia *et al.* 1986). A dsRNA of this size could encode five or six proteins of M_r 50000, one or more of which could decrease pathogenicity to a degree varying with the dsRNA coding sequences of different H strains. Most H strains have several dsRNA segments, some being deletions of the largest dsRNA whereas others are probably satellites. Such molecules could modulate the level of hypovirulence and are a further source of variation.

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Although dsRNA segments of European and North American H strains have similar terminal structures and have probably evolved from a common ancestor, they have evidently diverged considerably because no nucleotide sequence homology was detected between them by hybridization analysis (L'Hostis *et al.* 1985). However, it is likely that when the protein(s) responsible for hypovirulence is (are) identified and amino acid sequences determined, conserved functional domains will be revealed.

Attempts to use North American and European H-factors to control chestnut blight in North America have not met with widespread success. The ability of naturally occurring and introduced H-strains to spread in North America has been studied for only about 10 years whereas in Italy H-strains have been spreading for at least 36 years. It may therefore be too early to assess the situation in North America. However, possible lack of suitable vectors for spreading conidia of H-strains and the occurrence of large numbers of v-c groups in virulent isolates in North America are serious obstacles for blight control with H strains. In Connecticut, where natural spread of H-strains has not been observed, Anagnostakis (1983) classified 76 isolates in 14 forest lots of 1 acre† within Cockaponset State Forest into 35 different v-c groups. In tests of all possible combinations of 97 isolates, in pairs, all belonging to different v-c groups, transfer of the cytoplasmic determinants for curative morphology was efficient only between weakly barraging pairs that were a small proportion of the total (87 out of 4656). Possible ways in which vegetative compatibility reactions might be suppressed are considered in the next section.

(*d*) *Transmissible disease factors in Ophiostoma ulmi*

The current epidemics of Dutch elm disease which have devastated the elm populations of Europe and North America are caused by two races of the aggressive subgroup of the ascomycete fungus *Ophiostoma* (*Ceratocystis*) *ulmi* (reviewed by Brasier (1986*b*, 1987)). The North American (NAN) race, responsible for epidemics in the U.S.A. and Canada, is believed to have been imported into the U.K. during the mid 1960s and has since spread southwards and eastwards (Brasier 1979, 1987; Houston 1985). The Eurasian (EAN) race appears to have originated from central Europe or further east and has spread westwards across Europe (Brasier 1979, 1987). In some countries, e.g. Holland and Italy, both races are now present with evidence for EAN–NAN hybrids (Brasier 1986*c*). The EAN race has not been found in North America.

The non-aggressive subgroup of *O. ulmi* is now thought to be that first recorded in northwest Europe in 1918, spreading quickly into other areas including the U.S.A. It causes relatively mild disease on European elms, competes poorly against either race of the aggressive subgroups, and may eventually become extinct (Brasier 1986*b*, 1987). The EAN and NAN races of the aggressive subgroups are sufficiently similar that they may have evolved comparatively recently from a common progenitor, perhaps through geographical isolation (Brasier & Webber 1987) although they also show important developmental differences. More extensive genetic differences are found between the non-aggressive and aggressive subgroups reinforced by reproductive isolation, precluding the possibility that the latter has arisen from the former by a simple mutation (Brasier 1982, 1987).

The biological cycle of *O. ulmi* has distinct pathogenic and saprophytic phases (see

† 1 acre = 0.405 hectare = 4046.856 m².

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Webber & Brasier 1984; Brasier 1986*b*). The pathogenic phase is initiated by bark beetles (Coleoptera: Scolytidae), which carry asexual spores and ascospores and feed in crotches of twigs on healthy trees in spring and summer. After spore germination and a brief mycelial phase the fungus invades xylem where it replicates as budding yeast-like cells. A host reaction, probably stimulated at least in part by various toxins produced by the fungus, such as cerato-ulmin (Richards & Takai 1984) leads to blockage of the xylem and death of the tree.

The saprophytic phase occurs in the bark, extending from late summer and autumn to spring and early summer of the following year, and originates not only from spores introduced by beetles that colonize the bark for breeding but also from fungus growing outwards from xylem into the bark. The fungus grows as a three dimensional network of interacting hyphae (Brasier 1984) in successive phases of colonization and recolonization, with distinct sequences of conidia, synnemata and perithecia (Lea & Brasier 1983; Webber & Brasier 1984). In spring, newly emerging adult beetles carry spores which initiate new disease cycles in the xylem of healthy elm trees.

Webber & Brasier (1984) have shown that *O. ulmi* has two overlapping cycles, an alternating pathogenic–saprophytic (xylem–bark) cycle and a completely saprophytic (bark–bark) cycle with direct transfer of inocula from breeding gallery to breeding gallery. It is clear that in the bark there is considerable potential for interactions between genotypes for the generation of new genotypes by sexual reproduction.

Diseased (d-infected) isolates of *O. ulmi*, characterized by slow growth, abnormal 'amoeboid' morphology, impaired ability to produce sexual spores, impaired viability of conidia and the ability to transmit the genetic determinants of the disease (d-factors) to healthy recipients by hyphal anastomosis, were described by Brasier (1983). Transmission from d-infected donor mycelia to healthy recipient mycelia is often accompanied by pigment production and by physical changes in the colony of the recipients, termed d-reactions. Studies with nuclear and cytoplasmic markers showed that the determinant was a cytoplasmic component, and indicated that it could be transmitted independently of marked mitochondria (Brasier 1983, 1986*a*). Different d-factors have been designated d^1, d^2, etc., and can vary in their effects (Brasier 1986*a*).

Diseased isolates have been found in the NAN and EAN races of the aggressive sub-group and in the non-aggressive subgroup (Brasier 1986*a*). Multiple dsRNA segments have been found in all d-infected, and a considerable proportion of healthy, EAN and NAN isolates so far examined (Rogers *et al.* 1988*a*). As in other dsRNA-determined phenotypes it appears that the diseased phenotypes are caused by specific sequences in one or more dsRNA segments. For example, d^2-infected isolates contain 10 dsRNA segments ranging in size from 3500 nucleotides to 340 nucleotides and numbered from 1 to 10 in order of decreasing size (Rogers *et al.* 1986*a*). Transmission of the d^2-factor converted healthy to diseased phenotypes and conversion was accompanied by transmission of the 10 dsRNA segments. Transmission of the 10 dsRNA segments into conidia was efficient, but on the infrequent occasions when three or more dsRNA segments were lost, loss always included segments 4, 7 and 10 and the conidial isolates were healthy. Similar results were obtained after inoculating d^2-infected isolates into the xylem of young elm trees when the fungus multiplied in the yeast form. Recovered isolates that had lost segments 4, 7 and 10 were always healthy and it was concluded that the d^2-factor probably consisted of one or more of these three segments (Rogers *et al.* 1986*a*). Ascospore progeny from sexual crosses between healthy and diseased isolates or between two diseased isolates were

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always healthy (Brasier 1983, 1986*c*) and had lost all or most of the dsRNA segments of the parents (Rogers *et al.* 1986*a*, 1988*a*).

Although d-infected isolates survive in natural populations and are found at current epidemic fronts in Europe (Brasier 1986*a*), they have not yet had a major impact on the spread or severity of Dutch elm disease. The proportion of d-infected individuals will depend on a dynamic equilibrium established by the opposing rates of acquisition and loss of d-factors by individuals and by the relative rates of propagation and death of healthy and d-infected isolates. Factors determining such gains and losses will vary with the heterogeneity of populations and their locations with respect to epidemic fronts.

Most isolates in regional collections of *O. ulmi* have been found to belong to different v-c groups. The latter would be expected to reduce the efficiency of spread of d-factors by hyphal anastomosis in the saprophytic phase in the bark because in fully vegetatively incompatible reactions d-factor spread is restricted to only *ca.* 4% of pairings (Brasier 1984, 1986*a*). However, in some locations significant proportions of isolates belong to single predominant v-c groups, (termed v-c supergroups) so that the proportion of d-infected isolates is larger than normal. For example, at the current epidemic front in Poland, 47% of a sample of the *O. ulmi* population were in the same EAN v-c supergroup and 31% of these were severely d-infected (Brasier 1986*c*), and in more recent studies d-infection levels of 70% have been recorded in v-c supergroup samples (C. M. Brasier, unpublished observations).

The predominance of one v-c group in populations, e.g. at the current epidemic front in Portugal where more than 90% of the population belongs to the NAN v-c supergroup, could be due to selection for a high level of fitness (Brasier & Mitchell 1986). If so, the greater opportunity for d-factors to spread by hyphal anastomosis may be counteracted to some extent by a greater selection against d-infected members of the population.

Properties that may favour selection against d-infected individuals are reduced viability of conidia, slow hyphal growth, poor survival during flight of beetles and slower reproduction in the yeast phase (Brasier 1986*a*). The decreased fitness caused by d-infection is illustrated by the observation that infection requires at least 50000 conidia of a d^2-infected isolate but only 1000 conidia of a healthy isolate (Webber 1988).

Sexual reproduction generally results in loss of d-factors and generates new v-c types by recombination, thus decreasing the frequency of transmission of d-factors between individuals. Sporulation is reduced by d-infection, but ascospores are still produced fairly abundantly even when both parents are d-infected (Brasier 1986*a*).

Loss of d-factors can also occur during the yeast phase in the xylem (Brasier 1986*a*; Rogers *et al.* 1986*a*, 1988*a*). Such reversion may account in part for the observation that, if sufficient spores are injected into elm trees to initiate infections, d-infected isolates are not uniformly hypovirulent (Brasier 1986*a*). The frequency of loss of different d-factors during the yeast phase may vary and it is noteworthy that the d-infected isolates which are a significant proportion of isolates collected from the epidemic front in Poland (Brasier 1986*c*) were obtained from the xylem.

D-infections may become latent i.e. the d-factor and specific dsRNA segments are present but the d-phenotype is not expressed. A large proportion of the viable conidia of d^2-infected isolates are latently d-infected (Rogers *et al.* 1986*a*, *b*, 1988*b*); reversion to overt d-infection may occur spontaneously, by hyphal anastomosis with a healthy recipient (after which either the donor or recipient may show overt d-infection), by growth on certain media, or by a second cycle of conidiogenesis (after which a small proportion of the viable conidia are overtly

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d-infected). Because latently d-infected isolates would not be expected to be strongly selected against, latency is probably important in maintaining the frequency of d-factors in *O. ulmi* populations.

Brasier (1986*a*) has suggested that d-factors might exert their greatest effects in the post-epidemic phases when *O. ulmi* populations are much smaller. Debilitation of a proportion of a small population might reduce the population size below a critical threshold level. However, the increase in the frequency of sexual, as opposed to asexual, reproduction, which occurs in the post-epidemic phase (Brasier 1986*a*, *b*; Webber *et al.* 1988) would tend to decrease the frequency and therefore the effect of d-factors.

It appears that man will have to intervene if the proportions of overt d-infections necessary to control *O. ulmi* populations are to be achieved as suggested by Brasier (1986*a*) by breeding and releasing bark beetles carrying inocula of d-infected isolates, probably best done by targeting the beetles on the bark in the saprophytic phase of the cycle to maximize the spread of d-factors to the resident *O. ulmi* populations. If the new generation of beetles emerging from the breeding galleries carries predominantly propagules infected with particularly deleterious d-factors, then it may be incapable of establishing infection in feeding grooves of healthy elm trees. Thus common spore loads carried by the largest vector, *Scolytus scolytus*, are 250 to 5000 spores (Webber & Brasier 1984), whereas Webber (1988) has shown that at least 50000 conidia of a d^2-infected isolate are required for infection of the xylem, and spore viability could be further reduced during beetle flight (see above). Hence widespread transmission of d-factors to healthy individuals in the bark could be sufficient to interrupt the saprophytic–pathogenic cycle of *O. ulmi*. It has also been suggested that the transmission of d-factors in the short mycelial phase in feeding grooves would act as a 'back-up' to attenuate any healthy propagules that had escaped transmission in the bark (Brasier 1986*a*; Webber 1988).

If this interesting strategy is to be successful there are several problems to overcome.

(i) Transmission of d-factors to healthy individuals must be efficient. Although substantial proportions of individuals in some locations may belong to v-c supergroups the problem of vegetative incompatibility in the rest of populations must be solved. Brasier (1986*a*) suggested the use of isolates in v-c 'bridging-groups', which are partly compatible with a range of isolates that are completely incompatible with each other. Alternatively, d-factors could be introduced into a range of different isolates by direct transfection of protoplasts with isolated dsRNA by adapting methods used to infect *Gaeumannomyces graminis* var. *tritici* (Stanway & Buck 1984) and *Saccharomyces cerevisiae* (Sturley *et al.* 1987) with isolated dsRNA viruses. Because of the likely large number of v-c groups in *O. ulmi* (Brasier 1984) such approaches may not be sufficient. It will probably be necessary to find a way to suppress vegetative incompatibility reactions.

Although vegetative incompatibility is widespread in fungi (Lane 1981) and is well characterized genetically, virtually nothing is known about the molecular basis. In the slime mould *Physarum polycephalum* post-fusion vegetative incompatibility can be unidirectional or bidirectional (Lane & Carlile 1979). The strength of incompatibility reactions varied with the medium on which the strains were paired, ranging from death of fused plasmodia to mild reactions in which plasmodia survived with unilateral elimination of the nuclei of one strain. Such mild incompatible reactions would be unlikely to impede transmission of a cytoplasmic genetic element. Although the 'medium' on which *O. ulmi* grows in the wild obviously cannot be altered, the *P. polycephalum* observations show that vegetative incompatibility reactions can be modified.

A possible way of completely suppressing vegetative incompatibility reactions would be by

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the use of anti-sense messenger RNA (mRNA) (Benedetti *et al.* 1987) to block the expression of v-c genes. General methods for transformation of fungi are now available (Yoder *et al.* 1986) and v-c genes are potentially capable of being cloned. Hence it may be possible to transform selected *O. ulmi* isolates with DNA constructs allowing production of anti-sense mRNAs to the v-c genes of these isolates to suppress their expression. A particular target could be the *w* locus which is a major locus in v-c gene expression (Brasier 1984). Then d-factors could be introduced into these isolates, before their dissemination by bark beetles bred in captivity. Alternatively, genetically manipulated d-factors carrying anti-sense v-c genes may be produced because it is now possible to construct copy DNA (cDNA) clones of RNA viruses, manipulate them at the DNA level and transcribe the cDNA back into infectious RNA (Dawson *et al.* 1986). With this approach the coat protein gene of brome mosaic virus was replaced with the gene for chloramphenicol acetyltransferase (CAT). When this construct was introduced, with the other virus genome segments, the *CAT* gene was replicated and high levels of CAT mRNA and CAT were produced (French *et al.* 1986).

An exciting possibility, if hyphal fusion between isolates of the aggressive and non-aggressive subgroups could occur without causing a vegetative incompatibility reaction, is that isolates of the non-aggressive subgroup could be used to disseminate d-factors to populations of the aggressive subgroup.

(ii) The d-infected isolate(s) selected for control purposes should be so debilitated that beetle spore inocula cannot infect xylem of healthy elm trees, but retain sufficient viability for the initial inoculum to infect bark of diseased elms. Hence careful selection of d-factors will be necessary. Fortunately, a large pool of possible d-factors, which vary greatly in their effects, exists in the aggressive (NAN and EAN) and non-aggressive subgroups (Brasier 1986*a*). There is also limited evidence to suggest that d-factor from one subgroup may be more deleterious when transferred to another (Brasier 1983).

Little is known about the mode of action of d-factors. One effect of the d^2-factor appears to be reduction in cytochrome oxidase levels (Rogers *et al.* 1988*a*; Rogers 1987), but whether this is its only effect and whether other d-factors cause the same effect is unknown. If more could be learned about the mode of action of d-factors it may be possible to contruct genetically manipulated d-factors designed to debilitate their hosts to specific degrees.

(iii) The selected d-factor(s) should suppress the sexual stage of the fungus, although allowing a reasonable asexual reproduction. The ability of H-factors to suppress the sexual stage in *E. parasitica* clearly is important in the current predominance of H-strains in Italy. Widespread screening will be necessary to establish whether one or more d-factors with the desired properties exists in natural populations of *O. ulmi*.

Construction of genetically manipulated d-factors able to suppress the sexual stage is possible. Little is known of the molecular genetics of sexual reproduction in the filamentous fungi, but by analogy with asexual reproduction (conidiogenesis) in *Aspergillus nidulans* (Timberlake 1980), it is likely to be very complex. However, if the gene for one essential step in the sexual stage could be identified and cloned, the anti-sense mRNA approach could be used to suppress it.

It is interesting that in some fungi there is close association between sexual compatibility and vegetative incompatibility: for example in *Neurospora crassa* the mating type locus *A/a* appears to control both (Newmeyer *et al.* 1973), and in *Podospora anserina* the *mod* genes that suppress vegetative incompatibility also cause sterility (Boucherie *et al.* 1976). Perhaps a way could be

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found to suppress vegetative incompatibility and the sexual stage simultaneously in *O. ulmi*, although in the latter sexual compatibility and v-c appear not to be associated (Brasier 1984).

(iv) The selected d-infected isolate(s) should not switch to latent d-infection with great frequency. Although ability to switch to latency probably helps to ensure the survival of d-factors in natural populations, it would be a disadvantage for control purposes. Investigation of the molecular nature of the switch from overt to latent d-infection, and *vice versa*, may enable genetically manipulated d-factors incapable of switching to be constructed.

In conclusion, d-factors (naturally occurring or genetically manipulated) offer exciting possibilities for the control of Dutch elm disease and the re-establishment of the elm. However, many problems remain and much further research is needed before the potential of d-factors becomes a reality.

(*e*) *dsRNA-associated hypovirulence in other plant pathogenic fungi*

(i) *Helminthosporium victoriae*

Helminthosporium victoriae, which causes blight on oat cultivars with the Victoria-type of resistance to crown rust (*Puccinia coronata*), seriously decreased yields in most oat-growing areas of the U.S.A. in 1947 and 1948. Subsequently, Victoria-derived oat cultivars were abandoned in the major oat cropping areas, but continued to be grown in some of the southern states in the 1950s. Among cultures of *H. victoriae* newly isolated from oat crops in Louisiana, Lindberg (1959) encountered several stunted colonies, characterized by sectors at colony margins, collapse or lysis of aerial mycelium and almost complete inhibition of colony expansion. This 'disease' was also transmitted to healthy cultures by hyphal anastomosis and is probably caused by one of two dsRNA viruses commonly found in *H. victoriae* (Ghabrial 1986). The disease was transmitted by apparently healthy 'carrier' cultures which may be analogous to the latently d-infected isolates of *O. ulmi*. The oat crops from which diseased isolates were obtained did not suffer significant yield losses possibly because of spread of the virus in the *H. victoriae* population, because diseased isolates produced little of the toxin victorin and were much less pathogenic than normal isolates (Lindberg 1960).

(ii) *Rhizoctonia solani*

Rhizoctonia solani is a soil-borne pathogen of over 130 plant species (Baker 1970). Some isolates produce abnormal sectors giving cultures characterized by irregular morphology, few or no selerotia and an extremely reduced growth rate, a syndrome referred to as *Rhizoctonia* decline (Castanho & Butler 1978*a*). Diseased isolates of *R. solani* are weakly pathogenic and effective in reducing damping-off of sugar beet seedlings when applied to seed furrows of soil previously infected with a strongly pathogenic isolate (Castanho & Butler 1978*b*). Use of diseased isolates of *R. solani* is limited to short-term biological control because, unlike healthy isolates, they do not become established in either sterile or non-sterile soil, surviving for less than a month.

Control of healthy pathogenic strains by diseased strains of *R. solani* was considered to result from transmission of a cytoplasmic determinant from diseased to healthy strains. Diseased isolates contained three segments of dsRNA, whereas no dsRNA, or only traces of dsRNA segments, were detected in apparently healthy hyphal tip cultures from diseased isolates.

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Transmission of the dsRNA segments by hyphal anastomosis converted healthy hyphal tip cultures to the diseased phenotype (Castanho *et al.* 1978). However, no nuclear markers were used and therefore cytoplasmic transmission has not been proved unequivocally. In addition, re-examination by Finkler *et al.* (1985) of the above hyphal tip cultures revealed the same dsRNA segments as diseased isolates. McFadden *et al.* (1983) also found that hyphal tip cultures from a dsRNA-containing strain of *G. graminis* var. *tritici* contained very small quantities of dsRNA virus particles, detectable only by very sensitive immunological or hybridization methods. However, after 18 months and a number of subcultures, the numbers of virus particles had returned to those of the parent and were easily detected by standard methods. It is likely that the hyphal tip cultures of *R. solani* (Castanho *et al.* 1978) also retained low levels of dsRNA which subsequently increased. It is now clear that dsRNA segments are present in most, if not all, pathogenic strains of *R. solani* (Zanzinger *et al.* 1984; Finkler *et al.* 1985) so that the role of dsRNA, or specific segments of dsRNA, in *Rhizoctonia* decline, remains to be clarified. It is possible that the disease is caused by small linear DNA plasmids such as those reported in some slow growing, weakly pathogenic strains of *R. solani* (Hashiba *et al.* 1984), by defects in mitochondrial DNA, which cause senescence in *Podospora anserina*, the 'poky' and 'stopper' phenotypes of *Neurospora crassa*, and the 'ragged' phenotype of *Aspergillus amstelodami* (reviewed by Bockelmann *et al.* (1986)), or even by nuclear mutations. Even if cytoplasmic transmission can be proved, the prospects for biological control may be limited by the two types of vegetative incompatibility in *R. solani*. Hyphae of isolates belonging to different anastomosis groups cannot fuse so that cytoplasmic transmission is not possible. Even within anastomosis groups, post-fusion incompatibility, as in *E. parasitica* and *O. ulmi*, is common (Anderson 1984) and it is noteworthy that Castanho *et al.* (1978) were unable to transmit the genetic determinant of the disease to four other field isolates of the same anastomosis group.

Martini *et al.* (1978) claimed that determinants for pathogenicity in *R. solani* lie on a large DNA plasmid. More recently, Finkler *et al.* (1985) obtained evidence, based on transmission by hyphal anastomosis between genetically marked strains, that a cytoplasmic factor, suggested to lie on dsRNA segments, is required for pathogenicity. Field isolates, or hyphal tip isolates from virulent dsRNA-containing strains, which lacked dsRNA were hypovirulent. Hypovirulence in these isolates resulted from specific lack of a virulence factor rather than general degeneration of the isolates, as in *Rhizoctonia* decline (Castanho & Butler 1978*a*), because hypovirulent isolates grew in culture as fast as virulent isolates. Hypovirulent isolates of this type are being used successfully in Israel to protect plants from infection by virulent strains of *R. solani* (Y. Koltin, personal communication). In this situation successful biological control may depend *inter alia* on failure to transmit cytoplasmic virulence factors from virulent to hypovirulent isolates because of vegetative incompatibility barriers.

Further research is needed to clarify the role of cytoplasmic factors in the biology of *R. solani*. Such research should involve direct transformation with isolated dsRNA viruses or DNA plasmids to avoid uncertainties associated with transmission by hyphal anastomosis, compounded for this fungus by the possibility that isolates may be heterokaryotic (Anderson 1984).

(iii) *Gaeumannomyces graminis var. tritici*

Pathogenicity of the wheat take-all fungus is difficult to quantify because firstly the several methods of measurement may be influenced differently by environmental factors and may not

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give equivalent results (Asher 1981) and secondly isolates can readily lose their pathogenicity on storage (Chambers 1970; Naiki & Cook 1983). However, isolates from natural populations do vary in pathogenicity from strongly pathogenic to hypovirulent (Asher 1980), even when obtained from lesions (Asher 1978). Such pathogenic variation shows clear evidence of being under nuclear polygenic control (Blanch *et al.* 1981) although additional effects of cytoplasmic elements in a proportion of isolates cannot be discounted.

The dsRNA virus particles are common in both strongly and weakly pathogenic isolates (reviewed by Buck (1986*b*)) and attempts to establish correlations between the presence or absence of particles have failed (Rawlinson *et al.* 1973). However, Stanway (1985) found that a greater proportion of isolates with dsRNA segments had reduced pathogenicity compared with isolates with no dsRNA, suggesting that dsRNA segments in a proportion of isolates may carry determinants for hypovirulence. One freshly isolated field isolate that was weakly pathogenic in both short-term (seedling) and long-term (full season) tests contained nine dsRNA segments. Single conidial isolates from this isolate were either hypovirulent and retaining all the dsRNA segments of the parent, or virulent and dsRNA-free. Hence there is a good correlation between the presence of the dsRNA segments and hypovirulence. Although virulent conidial isolates readily produced perithecia and ascospores (*G. graminis* is homothallic), it has not proved possible to produce these from the hypovirulent isolates, suggesting that virulence and sexual reproduction are suppressed together, as in *E. parasitica*. This would be useful in using hypovirulent strains for biological control, because virus particles and dsRNA are frequently excluded during ascospore formation in *G. graminis* (Rawlinson *et al.* 1973; McFadden *et al.* 1983).

The problem of vegetative incompatibility, which is common in field isolates (Jamil *et al.* 1984) would still need to be solved. If vegetative incompatibility could be suppressed by using methods analogous to those suggested for *O. ulmi* it may be possible to transfer the dsRNA determinants for hypovirulence to avirulent or weakly pathogenic relatives of *G. graminis* var. *tritici*. *Phialophora* spp. with lobed hyphopodia is often found on cereal roots in the U.K., whereas *P. graminicola* is common in grasslands and is found on cereal crops when these follow grass leys or are infected with grass weeds (Deacon 1981). Such *Phialophora* spp., which had been transfected with dsRNA determinants for hypovirulence, might combine their intrinsic ability for cross-protection (see above) with the ability to convert pathogenic *G. graminis* var. *tritici* to hypovirulence by transmission of the dsRNA segments. Such isolates would have considerable advantages over hypovirulent *G. graminis* var. *tritici* for widespread distribution as biological control agents because they would not become pathogenic even if they lost the dsRNA segments.

(*f*) *Future prospects*

Cytoplasmic determinants for hypovirulence may be widespread among fungal plant pathogens. Their frequency may be low in populations of some fungi because of factors selecting directly against these determinants or against the hypovirulent isolates themselves. Searches for hypovirulence would be justified in a wide range of pathogens, but screening of several hundred isolates of a species may be necessary. Because the determinants for hypovirulence may lie on only a small fraction of the dsRNA segments or DNA plasmids found in populations of some pathogens, there is little value in attempting to establish correlations between the presence of dsRNA or DNA plasmids and hypovirulence. A more profitable

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approach would be to identify hypovirulent isolates, to establish cytoplasmic transmission and then identify the determinants.

Attempts to solve such problems as vegetative incompatibility, loss of determinants after sexual reproduction and latency, emphasize our ignorance of the molecular genetics, not only of plant pathogens, but of filamentous fungi in general. However, the use of recombinant DNA techniques to suppress vegetative incompatibility and the use of avirulent, close relatives of pathogens to deliver hypovirulence determinants to pathogen populations are attractive goals for the future.

3. Protection of plants from virulent strains of viruses by prior infection with mild strains

Infection of a plant with a virus can lead to a hypersensitive response (HR) which localizes the virus to a relatively small region of cells at the site of entry. This HR may induce rather non-specific localized or systemic resistance not only to a range of related and unrelated viruses, but also to other pathogens such as bacteria and fungi. Such resistance is caused by spread of host-induced factors to parts of the plant not carrying the virus (reviewed by Gianiazzi 1984; Ponz & Bruening 1986; Zaitlin & Hull 1987).

With many host–virus interactions the HR is not evoked and the virus infects the plant systemically. If the virus is a mild strain causing little disease, the plants become resistant specifically to pathogenic strains of the same virus. Such resistance, which is not host-induced and depends on the presence throughout the plant of the mild strain, has been called 'cross-protection'. Protection is not always complete but replication of the severe strain is usually depressed to the point where crop yields are not significantly reduced.

Potentially, any mild strain may protect against a severe strain of the same virus, but because of possible drawbacks (see later) the method has only been attempted for diseases which cause serious crop losses. Two diseases where cross-protection has succeeded are tomato mosaic and citrus tristeza.

Tomato mosaic virus, which is transmitted through seed and mechanically, is widespread. Tomatoes are grown annually from seed, and young plants can be protected by inoculation with mild virus strains by using high pressure spray guns. Protection against severe strains is usually good, yields of tomatoes are not significantly reduced by the mild virus strains and the method was used commercially for several years in Europe and Japan before being largely superseded by the use of resistant varieties (reviewed by Fulton (1986)).

Citrus tristeza virus is economically the most important citrus virus in the world (Bar-Joseph *et al.* 1981). The virus is transmitted by aphids, and spread so rapidly after its introduction from Africa into South America in the 1920s that the citrus industry in large areas of Argentina, Brazil and Uruguay was virtually wiped out in less than 20 years. A mild strain of the virus was first reported by Grant & Costa (1951) and, as a result of a U.S.A.–Brazil cooperation starting in 1961, many more have since been isolated and tested in a wide range of root stock – scion combinations for ability to protect against virulent strains of the virus. The most spectacular success has been with Pera sweet orange of which by 1980 more than eight million cross-protected trees had been planted in Brazil with very little failure of protection in successive clonal generations (Costa & Muller 1980). There are two main reasons for this success. First, the disease had caused such devastation that there was little to lose by testing

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mild strains on a large scale. Secondly, many mild strains were tested to find the few with sufficiently mild symptoms combined with good cross protection.

Possible applications of cross-protection for the control of several diseases of perennial plants, such as cacao swollen shoot, papaya ringspot, avocado sun blotch and several virus diseases of stone and pome fruits, have been reviewed by Fulton (1986). However, despite the few notable successes, cross-protection has not been widely used for the following reasons.

First, it can be difficult and expensive to inoculate plants with the mild strains. This is not such a problem for perennial plants or for plants propagated vegetatively, because the virus, once introduced, persists over long periods and many clonal propagations. However, it has generally been considered impracticable for field crops of annual plants when the virus must be applied to each plant in each crop. Growers prefer to use resistant cultivars when these are available, or to use insecticidal sprays to kill insect vectors of viruses.

Secondly, there is justifiable caution about the widespread distribution in field crops of mild strains of viruses, which may give severe synergistic reactions in mixed infections with unrelated viruses, may revert to virulence or may cause severe disease if transmitted to other crops by insect vectors (Fulton 1986).

As a result of recent advances in molecular biology and recombinant DNA technology solutions to some, or all, of these problems can now be envisaged. First, construction of mild strains of viruses incapable of reverting to virulence or of vector transmission is feasible. Secondly, it might be possible to protect plants by expression of a single viral gene rather than by infection with intact virus.

Mild strains of viruses have traditionally been obtained directly from nature, by culturing infected tissue at supra-optimal temperatures for replication of the pathogenic virus strains or by nitrous acid mutagenesis (Fulton 1986). Recently, the nucleotide sequence of an attenuated strain of tomato mosaic virus has been determined and compared with that of the parent pathogenic strain (Nishiguchi *et al.* 1985). Of the ten base-substitutions found in the attenuated strain, seven occurred in the third base of codons and did not alter amino acids. The remaining three were in the common reading frame of 130 kDa and 180 kDa proteins which may be involved in viral RNA replication. Hence the mutations may reduce the capacity of the virus to replicate its RNA genome. Techniques of *in vitro* mutagenesis are now available (Smith 1985) which enable essentially any base or group of bases to be altered in a nucleic acid molecule. Hence mild virus strains could be constructed by *in vitro* mutagenesis of the genome of pathogenic strains and the effects of introducing deletions into the genome, which could not easily revert to the parental sequence, could be studied. Similarly, deletions could be made in virus genes essential for vector transmission. Non-aphid transmissible mutants of cauliflower mosaic virus with deletions in gene *II*, which encodes an aphid transmission protein, occur naturally and have also been constructed by genetic manipulation (Woolston *et al.* 1983).

The use of genetically manipulated mild strains would probably be limited to perennials and vegetatively propagated plants, because of the problems of application to annual crops outlined above. The alternative approach of expressing single virus genes in plants potentially has more general applicability. The mechanisms of cross-protection are unknown, but the following hypotheses have been put forward (reviewed by Zinnen & Fulton (1986)), some of which enable candidate genes to be identified and tested.

Coat protein of the pre-infecting strain could prevent uncoating of the challenge virus. The observation that extraneous tobacco mosaic virus (TMV) coat protein inhibited the *in vitro*

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co-translational disassembly of pre-swollen TMV (Wilson & Watkins 1986) is consistent with this hypothesis. Recently Abel *et al.* (1986) introduced a chimeric gene containing a cloned cDNA of the TMV coat-protein gene into the genome of tobacco cells by using a Ti plasmid vector. The resultant transgenic plants expressed the TMV coat protein as a nuclear trait. When challenged with TMV the symptoms in transgenic plants were delayed and 10–60% of the plants failed to develop symptoms, results similar to those reported for cross-protection of TMV in tomato (Cassells & Herrick 1977). Studies of virus replication indicated that symptom-less plants are not infected, showing that plants can be genetically transformed for resistance. Transgenic plants were not resistant to infection with TMV RNA (R. N. Beachy, unpublished observation quoted in Zaitlin & Hull (1987)) suggesting that blocking of virus uncoating is indeed one mechanism of resistance to virus infection.

Blocking of virus uncoating is clearly not the only mechanism of cross-protection that can be engendered by coat protein-defective or coat-less mutants of TMV (Zaitlin 1976; Sarkar & Smitamana 1981) and by viroids that are naked RNA molecules (Niblett *et al.* 1978). Palukaitis & Zaitlin (1984) suggested a more general mechanism called negative-strand capture. In the replication of positive-strand RNA viruses, which compose the majority of plant viruses, an RNA-dependent RNA polymerase catalyses the synthesis of a complementary negative strand by using the positive strand as a template; more positive strands are then synthesized by using the negative strands as templates. Palukaitis & Zaitlin (1984) proposed that positive strands of a pre-infecting mild virus strain would hybridize with the first negative strands to be produced by a related challenge virus and prevent them from acting as templates to produce more positive strands.

If cDNAs to viral RNA were linked to suitable promoters and integrated into plant genomes so that the plants produced either a whole negative strand or part of a positive strand, the replication of a challenge virus RNA could be inhibited by positive- or negative-strand capture respectively. The method could apply equally well to viroids.

Positive-strand capture is similar in principle to the use of anti-sense RNA to inhibit gene expression (Benedetti *et al.* 1987) and could suppress virus replication by preventing the synthesis of an essential viral protein. However, binding of RNA-dependent RNA polymerase to recognition sequences could also be inhibited by expression by the host plant of sequences complementary to the 3′ ends of positive or negative strands. Use of positive- or negative-strand capture or anti-sense RNA is being investigated in several laboratories and information on the feasibility of this approach in producing virus-resistant plants is expected soon; mRNA-interfering complementary RNA (anti-sense RNA) is known to inhibit RNA bacteriophage replication in *Escherichia coli*, plaque formation being reduced by up to 98% (Hirashima *et al.* 1986).

Symptoms of disease development can be modified by satellite RNAs (sat-RNAs) in a virus culture. Satellite RNAs only replicate in the presence of a helper virus and are encapsidated by coat protein encoded by the helper virus; sat-RNAs have little sequence homology with the helper virus and are not required for its replication. Although some sat-RNAs increase virus symptoms, attenuation is more common (Francki 1985). It has been reported that pre-infection with a cucumber mosaic virus (CMV) strain containing a benign sat-RNA, protected against CMV and increased yield of pepper plants (Tien & Chang 1984). Also, infection of tomato plants with a CMV strain containing a benign sat-RNA protected against the effects of a CMV culture containing a virulent sat-RNA (Jacquemond 1982).

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Baulcombe *et al.* (1986) introduced cDNA dimers of a benign CMV sat-RNA into the genome of tobacco plants by using an expression vector. RNA transcripts of the expected size were detected in transformed plants but there was no evidence for production or replication of unit-length sat-RNA and plants showed no symptoms. However, after infection of plants with CMV, formation and replication of sat-RNA was observed. This suggested that plants could be protected from disease symptoms caused by CMV by expressing cDNAs of sat-RNAs from the nuclear genome. However, although sat-RNAs reduce replication of their helper virus they cannot suppress it completely because of their dependence on helper virus replication. Hence they make plants tolerant of, rather than resistant to, virus infection.

In conclusion, the availability of recombinant DNA techniques to manipulate genetically both viruses and their plant hosts has led to a resurgence of interest in cross-protection. The construction of genetically engineered plants that are resistant to viral infection by the mechanisms involved in cross-protection, but without the possible hazards of the widespread dissemination of infectious agents in the environment, can now be seen as a desirable and feasible goal for the future. The use of such resistant plants may not be seen by some as biological control *sensu stricto* because it does not involve control by a third organism, but it fits nicely into the broader definition of biological control (of a plant pathogen) of Cook & Baker (1983) i.e. 'reduction in the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man'. Nevertheless, it is clear that investigations of biological control have opened up several novel approaches for the control of plant virus diseases.

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References

Abel, P. P., Nelson, R. S., De, B., Hoffman, N., Rogers, S. G., Fraley, R. T. & Beachy, R. N. 1986 Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science, Wash.* **232**, 738–743.

Anagnostakis, S. L. 1982 Biological control of chestnut blight. *Science, Wash.* **215**, 466–471.

Anagnostakis, S. L. 1983 Conversion to curative morphology in *Endothia parasitica* and its restriction by vegetative compatibility. *Mycologia* **75**, 777–780.

Anagnostakis, S. L. 1984 The mycelial biology of *Endothia parasitica*. II. Vegetative incompatibility. In *The ecology and physiology of the fungal mycelium* (ed. D. H. Jennings & A. D. M. Rayner), pp. 499–507. Cambridge University Press.

Anderson, N. A. 1984 Variation and heterokaryosis in *Rhizoctonia solani*. In *The ecology and physiology of the fungal mycelium* (ed. D. H. Jennings & A. D. M. Rayner), pp. 367–382. Cambridge University Press.

Asher, M. J. C. 1978 Isolation of *Gaeumannomyces graminis* var. *tritici* from roots. *Trans. Br. mycol. Soc.* **71**, 322–325.

Asher, M. J. C. 1980 Variation in pathogenicity and cultural characters in *Gaeumannomyces graminis* var. *tritici*. *Trans. Br. mycol. Soc.* **75**, 213–220.

Asher, M. J. C. 1981 Pathogenic variation. In *Biology and control of take-all* (ed. M. J. C. Asher & P. J. Shipton), pp. 199–218. London: Academic Press.

Baker, K. F. 1970 Types of *Rhizoctonia* diseases and their occurrence. In *Rhizoctonia solani: biology and pathology* (ed. J. R. Parmeter), pp. 125–148. Los Angeles: University of California Press.

Bar-Joseph, M., Roistacher, C. N., Garnsey, S. M. & Gumpf, D. J. 1981 A review on tristeza, an ongoing threat to citriculture. *Proc. int. Soc. Citriculture* **1**, 419–422.

Baulcombe, D. C., Saunders, G. R., Bevan, M. W., Mayo, M. A. & Harrison, B. D. 1986 Expression of biologically active viral satellite RNA from the nuclear genome of transformed plants. *Nature, Lond.* **321**, 446–449.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Bazzigher, G., Kanzler, E. & Kubler, T. 1981 Irreversible pathogenitatsverminderung bei *Endothia parasitica* durch ubertragbare Hypovirulenz. *Eur. J. Forest Path.* **11**, 358–369.

Benedetti, A. D., Pytel, B. A. & Baglioni, C. 1987 Loss of (2′-5′) oligoadenylate synthetase activity by production of antisense RNA results in lack of protection by interferon from viral infections. *Proc. natn. Acad. Sci. U.S.A.* **84**, 658–662.

Berthelay-Sauret, S. 1973 Utilisation de mutants auxotrophes dans les recherches sur le déterminisme de 'l'hypovirulence exclusive'. *Annls Pytopath.* **5**, 318. (Abstract.)

Biraghi, A. 1953 Ulterion notizie sulla resistenze di *Castanea sativa* Mill. nei confronti de *Endothia parasitica* (Murr.) And. *Boll. Staz. Patol. veg. Roma* **11**, 149–157.

Blanche, P. A., Asher, M. J. C. & Burnett, J. H. 1981 Inheritance of pathogenicity and cultural character in *Gaeumannomyces graminis* var *tritici*. *Trans. Br. mycol Soc.* **71**, 367–373.

Bockelmann, B., Osiewacz, H. D., Schmidt, F. R. & Schulte, E. 1986 Extrachromosomal DNA in fungi-organisation and function. In *Fungal virology* (ed. K. W. Buck), pp. 237–283. Boca Raton, Florida: CRC Press.

Boucherie, H., Begueret, J. & Bernet, J. 1976 The molecular mechanisms of protoplasmic incompatibility and its relationship to the formation of protoperithecia in *Podospora anserina*. *J. gen. Microbiol.* **92**, 59–66.

Bozarth, R. F. 1977 Biophysical and biochemical characterization of virus-like particles containing a high molecular weight dsRNA from *Helminthosporium maydis*. *Virology* **80**, 149–157.

Brasier, C. M. 1979 Dual origin of recent Dutch elm disease epidemics in Europe. *Nature, Lond.* **281**, 78–79.

Brasier, C. M. 1982 Genetics of pathogenicity in *Ceratocystis ulmi* and its significance for elm breeding. In *Resistance to diseases and pests in forest trees* (ed. H. M. Heybrock, B. R. Stephan & K. von Weissenberg), pp. 224–235. Wageningen: Pudoc.

Brasier, C. M. 1983 A cytoplasmically transmitted disease of *Ceratocystis ulmi*. *Nature, Lond.* **305**, 220–223.

Brasier, C. M. 1984 Inter-mycelial recognition systems in *Ceratocystis ulmi*: their physiological properties and ecological importance. In *The ecology and physiology of the fungal mycelium* (ed. D. H. Jennings & A. D. M. Rayner), pp. 451–497. Cambridge University Press.

Brasier, C. M. 1986*a* The d-factor in *Ceratocystis ulmi* – its biological characteristics and implications for Dutch elm disease. In *Fungal virology* (ed. K. W. Buck), pp. 177–208. Boca Raton, Florida: CRC Press.

Brasier, C. M. 1986*b* The population biology of Dutch elm disease: the principal features and implications for other host-pathogen systems. *Adv. Pl. Path.* **5**, 53–118.

Brasier, C. M. 1986*c* Dutch elm disease – *Ophiostoma* (*Ceratocystis*) *ulmi*. The emergence of EAN and NAN hybrids in Europe. In *Report on forest research 1986*, p. 37. London: HMSO.

Brasier, C. M. 1987 Recent genetic changes in the *Ophiostoma ulmi* population: the threat to the future of the elm. In *Populations of plant pathogens: their dynamics and genetics* (ed. M. S. Wolfe & C. E. Caten), pp. 213–226. Oxford: Blackwell Scientific Publications.

Brasier, C. M. & Mitchell, A. G. 1986 Dutch elm disease – *Ophiostoma* (*Ceratocystis*) *ulmi*. Vegetative compatibility 'super-groups' in *O. ulmi*. In *Report on forest research 1986*, p. 38. London: HMSO.

Brasier, C. M. & Webber, J. F. 1987 Recent advances in Dutch elm disease research: host, pathogen and vector. In *Advances in practical arboriculture* (ed. D. Patch), pp. 166–179. (*Bull. For. Commn, Lond.* **65**.)

Buck, K. W. 1986*a* Fungal virology – an overview. In *Fungal virology* (ed. K. W. Buck), pp. 1–84. Boca Raton, Florida: CRC Press.

Buck, K. W. 1986*b* Viruses of the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*. In *Fungal virology* (ed. K. W. Buck), pp. 221–236. Boca Raton, Florida: CRC Press.

Cassells, A. C. & Herrick, C. 1977 Cross protection between mild and severe strains of tobacco mosaic virus in doubly inoculated tomato plants. *Virology* **78**, 253–260.

Castanho, B. & Butler, E. E. 1978*a* *Rhizoctonia* decline: a degenerative disease of *Rhizoctonia solani*. *Phytopathology* **68**, 1505–1510.

Castanho, B. & Butler, E. E. 1978*b* *Rhizoctonia* decline: studies on hypovirulence and potential use in biological control. *Phytopathology* **68**, 1511–1514.

Castanho, B., Butler, E. E. & Shepherd, R. J. 1978 The association of double-stranded RNA with *Rhizoctonia decline*. *Phytopathology* **68**, 1515–1518.

Chambers, S. C. 1970 Pathogenic variation in *Ophiobolus graminis*. *Aust. J. biol. Sci.* **23**, 1099–1103.

Cook, R. J. & Baker, K. F. 1983 *The nature and practice of biological control of plant pathogens*. (539 pages.) St Paul, Minnesota: The American Phytopathological Society.

Costa, A. S. & Muller, G. W. 1980 Tristeza control by cross protection: a U.S.–Brazil cooperative success. *Pl. Dis.* **64**, 538–541.

Dawson, W. O., Beck, D. L., Knorr, D. A. & Grantham, G. L. 1986 cDNA cloning of the complete genome of tobacco mosaic virus and production of infectious transcripts. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1832–1836.

Day, P. R. 1981 Fungal virus populations in corn smut from Connecticut. *Mycologia* **73**, 379–391.

Day, P. R. & Dodds, J. A. 1979 Viruses of plant pathogenic fungi. In *Viruses and plasmids of fungi* (ed. P. A. Lemke), pp. 201–238. New York: Marcel Dekker Inc.

Day, P. R., Dodds, J. A., Elliston, J. E., Jaynes, R. A. & Anagnostakis, S. L. 1977 Double-stranded RNA in *Endothia parasitica*. *Phytopathology* **67**, 1393–1396.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Deacon, J. W. 1981 Ecological relationships with other fungi: competitors and hyperparasites. In *Biology and control of take-all* (ed. M. J. C. Asher & P. J. Shipton), pp. 75–101. London: Academic Press.
Elliston, J. E. 1982 Hypovirulence. *Adv. Pl. Path.* **1**, 1–33.
Elliston, J. E. 1985 Characteristics of dsRNA-free and dsRNA-containing strains of *Endothia parasitica* in relation to hypovirulence. *Phytopathology* **75**, 151–158.
Finkler, A., Kolton, Y., Barash, I., Sneh, B. & Pozmak, D. 1985 Isolation of a virus from virulent strains of *Rhizoctonia solani*. *J. gen. Virol.* **66**, 1221–1232.
Francki, R. I. B. 1986 Plant virus satellites. *A. Rev. Microbiol.* **39**, 151–174.
French, R., Janda, M. & Ahlquist, P. 1986 Bacterial gene inserted in an engineered RNA virus: efficient expression in monocotyledonous plant cells. *Science, Wash.* **231**, 1294–1297.
Fulbright, D. W. 1984 Effect of eliminating dsRNA in hypovirulent *Endothia parasitica*. *Phytopathology* **74**, 722–724.
Fulbright, D. W., Weidlich, W. H., Haufler, K. Z., Thomas, C. S. & Paul, C. P. 1983 Chestnut blight and recovering American chestnut trees in Michigan. *Can. J. Bot.* **61**, 3164–3171.
Fulton, R. W. 1986 Practices and precautions in the use of cross-protection for plant virus disease control. *A. Rev. Phytopath.* **24**, 67–81.
Ghabrial, S. A. 1986 A transmissible disease of *Helminthosporium victoriae* – evidence for a viral etiology. In *Fungal virology* (ed. K. W. Buck), pp. 163–176. Boca Raton, Florida: CRC Press.
Gianinazzi, S. 1984 Genetic and molecular aspects of resistance induced by infections or chemicals. In *Plant–microbe interactions: molecular and genetic perspectives*, vol. 1 (ed. T. Kosuge & E. N. Nester), pp. 321–342. New York: Macmillan.
Grant, T. J. & Costa, A. S. 1951 A mild strain of the tristeza virus of citrus. *Phytopathology* **41**, 114–122.
Grente, J. 1965 Les formes hypovirulentes d'*Endothia parasitica* et les espoirs de lutte contre le chancre du chataigner. *C. r. hebd. Séanc. Acad. Agri. Fr.* **51**, 1033–1037.
Grente, J. 1981 Les variants hypovirulents de l'*Endothia parasitica* et la lutte biologique contre le chancre du chataigner. Ph.D. thesis, Université de Bretagne Occidentale, Brest.
Grente, J. & Sauret, S. 1969 L'hypovirulence exclusive phenomene orignal en pathologie vegetale. *C. r. hebd. Séanc. Acad. Sci., Paris* D **268**, 2347–2350.
Hansen, D. R., Van Alfen, N. K., Gillies, K. & Powell, W. A. 1985 Naked dsRNA associated with hypovirulence of *Endothia parasitica* is packaged in fungal vesicles. *J. gen. Virol.* **66**, 2605–2614.
Hashiba, T., Homma, Y., Hyakumachi, M. & Matsuda, I. 1984 Isolation of a DNA plasmid in the fungus *Rhizoctonia solani*. *J. gen. Microbiol.* **130**, 2067–2070.
Hebard, F. V., Griffin, G. J. & Elkins, J. R. 1984 Developmental histopathology of cankers incited by hypovirulent and virulent isolates of *Endothia parasitica* on susceptible and resistant chestnut trees. *Phytopathology* **74**, 140–149.
Hirashima, A., Sawaki, S., Inokuchi, Y. & Inouye, M. 1986 Engineering of the mRNA-interfering complementary RNA immune system against viral infection. *Proc. natn. Acad. Sci. U.S.A.* **83**, 7726–7730.
Hiremath, S., L'Hostis, B. L., Ghabrial, S. A. & Rhoads, R. E. 1986 Terminal structure of hypovirulence-associated dsRNAs in the chestnut blight fungus *Endothia parasitica*. *Nucl. Acids Res.* **14**, 9877–9896.
Houston, D. R. 1985 Spread and increase of *Ceratocystis ulmi* with cultural characteristics of the aggressive strain in north eastern North America. *Pl. Dis.* **69**, 677–680.
Jacquemond, M. 1982 The phenomena of interferences between the two types of satellite RNA of cucumber mosaic virus: protection of tomato plants against lethal necrosis. *C. r. hebd. Séanc. Acad. Sci., Paris* C **294**, 991–994.
Jamil, N., Buck, K. W. & Carlile, M. J. 1984 Sequence relationships between virus double-stranded RNA from isolates of *Gaeumannomyces graminis* in different vegetative compatibility groups. *J. gen. Virol.* **65**, 1741–1747.
Koltin, Y. 1986 The killer systems of *Ustilago maydis*. In *Fungal virology* (ed. K. W. Buck), pp. 109–141. Boca Raton, Florida: CRC Press.
Koltin, Y. & Day, P. R. 1975 The specificity of *Ustilago maydis* killer proteins. *Appl. Microbiol.* **30**, 694–696.
Lane, E. B. 1981 Somatic incompatibility in fungi and myxomycetes. In *The fungal mycelium* (ed. K. Gull & S. G. Oliver), pp. 239–258. Cambridge University Press.
Lane, E. B. & Carlile, M. J. 1979 Post-fusion somatic incompatibility in plasmodia of *Physarum polycephalum*. *J. Cell Sci.* **35**, 339–354.
Lea, J. & Brasier, C. M. 1983 A fruiting succession in *Ceratocystis ulmi* and its role in Dutch elm disease. *Trans. Br. mycol. Soc.* **80**, 381–387.
L'Hostis, B., Hiremath, S. T., Rhoads, R. E. & Ghabrial, S. A. 1985 Lack of sequence homology between double-stranded RNA from European and American strains of *Endothia parasitica*. *J. gen. Virol.* **66**, 351–355.
Lindberg, G. D. 1959 A transmissible disease of *Helminthosporium victoriae*. *Phytopathology* **49**, 29–32.
Lindberg, G. D. 1960 Reduction in pathogenicity and toxin production in diseased *Helminthosporium victoriae*. *Phytopathology* **50**, 457–460.
Martini, G., Grimaldi, G. & Guardiola, I. 1978 Extrachromosomal DNA in phytopathogenic fungi. In *Genetic engineering* (ed. H. W. Boyer & S. Nicosia), pp. 197–200. Amsterdam: Elsevier/North-Holland.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

McFadden, J. J. P., Buck, K. W. & Rawlinson, C. J. 1983 Infrequent transmission of double-stranded RNA virus particles but absence of DNA proviruses in single ascospore cultures of *Gaeumannomyces graminis*. *J. gen. Virol.* **64**, 927–937.

Mitemperhger, L. 1978 The present status of chestnut blight in Italy. In *Proceedings American chestnut symposium* (ed. W. L. MacDonald), pp. 34–37. Morgantown: West Virginia University Books.

Naiki, T. & Cook, R. J. 1983 Factors in loss of pathogenicity in *Gaeumannomyces graminis* var. *tritici*. *Phytopathology* **73**, 1652–1656.

Newmeyer, D., Howe, H. B. & Galeazzi, D. R. 1973 A search for complexity at the mating type locus of *Neurospora crassa*. *Can. J. Genet. Cytol.* **15**, 577–585.

Niblett, C. L., Dickson, E., Fernow, K. H., Horst, R. K. & Zaitlin, M. 1978 Cross protection among four viroids. *Virology* **91**, 198–203.

Nishiguchi, M., Kikuchi, S., Kiho, Y., Ohno, T., Meshi, T. & Okada, Y. 1985 Molecular basis of plant viral virulence: the complete nucleotide sequence of an attenuated strain of tobacco mosaic virus. *Nucl. Acids Res.* **13**, 5585–5590.

Palukaitis, P. & Zaitlin, M. 1984 A model to explain the 'cross-protection' phenomenon shown by plant viruses and viroids. In *Plant–microbe interactions: molecular and genetic perspectives* (ed. T. Kosuge & E. N. Nester), vol. 1, pp. 420–429. New York: Macmillan.

Ponz, F. & Bruening, G. 1986 Mechanisms of resistance to plant viruses. *An. Rev. Phytopath.* **24**, 355–381.

Rawlinson, C. J., Hornby, D., Pearson, V. & Carpenter, J. M. 1973 Virus-like particles in the take-all fungus, *Gaeumannomyces graminis*. *Ann. appl. Biol.* **74**, 197–209.

Richards, W. C. & Takai, S. 1984 Characterization of the toxicity of *Cerato-ulmi*, the toxin of Dutch elm disease. *Can. J. Pl. Path.* **6**, 291–298.

Rogers, H. J. 1987 The molecular nature of cytoplasmically inherited disease factors in *Ophiostoma ulmi*. Ph.D. thesis, University of London.

Rogers, H. J., Buck, K. W. & Brasier, C. M. 1986*a* Transmission of double-stranded RNA and a disease-factor in *Ophiostoma ulmi*. *Pl. Path.* **35**, 277–287.

Rogers, H. J., Buck, K. W. & Brasier, C. M. 1986*b* The molecular nature of the d-factor in *Ceratocystis ulmi*. In *Fungal virology* (ed. K. W. Buck), pp. 209–220. Boca Raton, Florida: CRC Press.

Rogers, H. J., Buck, K. W. & Brasier, C. M. 1988*a* Double-stranded RNA in diseased isolates of the aggressive subgroup of the Dutch elm disease fungus, *Ophiostoma ulmi*. In *Viruses of fungi and simple eukaryotes* (ed. M. J. Leibowitz & Y. Koltin). New York: Marcel Dekker Inc. (In the press.)

Rogers, H. J., Buck, K. W. & Brasier, C. M. 1988*b* The d^2-factor in *Ophiostoma ulmi*: expression and latency. In *Biology and molecular biology of plant–pathogen interactions* (ed. J. A. Bailey), pp. 393–400. Berlin: Springer-Verlag. (In the press.)

Sarkar, S. & Smitamana, P. 1981 A proteinless mutant of tobacco mosaic virus: evidence against the role of a viral coat protein for interference. *Molec. gen. Genet.* **184**, 158–159.

Shelbourn, S. L., Day, P. R. & Buck, K. W. 1987 Relationships and functions of virus double-stranded RNA in a killer strain of *Ustilago maydis*. *Abstracts of the 7th International Congress on Virology, Edmonton, 9–14 August, 1987*.

Smith, M. 1985 *In vitro* mutagenesis. *A. Rev. Genet.* **19**, 423–462.

Stanway, C. A. 1985 Double-stranded RNA viruses and pathogenicity of the wheat take-all fungus *Gaeumannomyces graminis* var. *tritici*. Ph.D. thesis, University of London.

Stanway, C. A. & Buck, K. W. 1984 Infection of protoplasts of the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*, with double-stranded RNA viruses. *J. gen. Virol.* **65**, 2061–2065.

Sturley, S. L., El-Sherbeini, M. & Bostian, K. A. 1988 The yeast killer virus as a secretion vector. In *Viruses of fungi and simple eukaryotes* (ed. Y. Koltin & M. J. Leibowitz). New York: Marcel Dekker. (In the press.)

Tartaglia, J., Paul, C. P., Fulbright, D. W. & Nuss, D. L. 1986 Structural properties of double-stranded RNAs associated with biological control of chestnut blight fungus. *Proc. natn. Acad. Sci. U.S.A.* **83**, 9109–9113.

Tien, P. & Chang, X. H. 1984 Vaccination of pepper with cucumber mosaic virus isolates attenuated with a satellite RNA. *Abstracts of the 6th International Congress on Virology, Sendai, Japan, September 1984*, p. 379.

Timberlake, W. E. 1980 Developmental gene regulation in *Aspergillus nidulans*. *Devl Biol.* **78**, 497–510.

Turchetti, T. 1978 Some observations on the 'hypovirulence' of chestnut blight in Italy. In *Proceedings of the American chestnut symposium* (ed. W. L. MacDonald), pp. 92–94. Morgantown: West Virginia University Agricultural Experiment Station.

Turchetti, T. 1979 Prospettive di lotta biologica in alcune malattie di piante forestali. *Infme fitopatol.* **29**, 7–15.

Van Alfen, N. K. 1982 Biology and potential for disease control of hypovirulence of *Endothia parasitica*. *A. Rev. Phytopath.* **20**, 349–362.

Van Alfen, N. K. 1986 Hypovirulence of *Endothia* (*Cryphonectria*) *parasitica* and *Rhizoctonia solani*. In *Fungal virology* (ed. K. W. Buck), pp. 143–162. Boca Raton, Florida: CRC Press.

Van Alfen, N. K., Jaynes, R. A., Anagnostakis, S. L. & Day, P. R. 1975 Chestnut blight: biological control by transmissible hypovirulence in *Endothia parasitica*. *Science, Wash.* **189**, 890–891.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Van Alfen, N. K., Jaynes, R. A. & Bowman, J. T. 1978 Stability of *Endothia parasitica* hypovirulence in culture. *Phytopathology* **68**, 1075–1079.
Vidaver, A. K. 1976 Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. *A. Rev. Phytopathol.* **14**, 451–465.
Webber, J. F. 1988 The influence of the d²-factor on survival and infection by the Dutch elm disease pathogen *Ophiostoma ulmi*. *Pl. Path.* (In the press.)
Webber, J. F. & Brasier, C. M. 1984 The transmission of Dutch elm disease: a study of the processes involved. In *Invertebrate–microbial interactions* (ed. J. M. Anderson, A. D. M. Rayner & D. W. H. Walton), pp. 271–306. Cambridge University Press.
Webber, J. F., Brasier, C. M. & Mitchell, A. G. 1988 The role of the saprophytic phase in Dutch elm disease. In *Fungal infestation of plants: establishment, progress and outcome of infection* (ed. G. F. Pegg & P. G. Ayres). Cambridge University Press. (In the press.)
Wigderson, M. & Koltin, Y. 1982 Dual toxin specificities and the exclusion relations among the *Ustilago* dsRNA viruses. *Curr. Genet.* **5**, 127–136.
Wilson, T. M. A. & Watkins, P. A. C. 1986 Influence of exogenous viral coat protein on the cotranslational disassembly of tobacco mosaic virus particles *in vitro*. *Virol.* **149**, 132–135.
Wong, P. T. W. 1981 Biological control by cross-protection. In *Biology and control of take-all* (ed. M. J. C. Asher & P. J. Shipton), pp. 417–431. London: Academic Press.
Woolston, C. J., Covey, S. N., Penswick, J. R. & Davies, J. W. 1983 Aphid transmission and a polypeptide are specified by a defined region of the cauliflower mosaic virus genome. *Gene* **23**, 15–23.
Yoder, O. C., Weltring, K., Turgeon, B. G., Garber, R. C. & Van Etten, H. D. 1986 Technology for molecular cloning of fungal virulence genes. In *Biology and molecular biology of plant–pathogen interactions* (ed. J. A. Bailey), pp. 371–384. Berlin: Springer-Verlag.
Zaitlin, M. 1976 Viral cross protection: more understanding is needed. *Phytopathology* **66**, 382–383.
Zaitlin, M. & Hull, R. 1987 Plant virus–host interactions. *A. Rev. Pl. Physiol.* **38**, 291–315.
Zanzinger, D. H., Bandy, B. P. & Tavantzis, S. M. 1984 High frequency of finding double-stranded RNA in naturally occurring isolates of *Rhizoctonia solani*. *J. gen. Virol.* **65**, 1601–1605.
Zinnen, T. M. & Fulton, R. W. 1986 Cross-protection between sunn-hemp mosaic and tobacco mosaic viruses. *J. gen. Virol.* **67**, 1679–1687.

Discussion

R. J. Cook (*United States Department of Agriculture Agricultural Research Service, Washington State University, Pullman, U.S.A.*). Would Professor Buck please define the term 'hypovirulence'? Specifically, is the term to be limited to a transmissible trait or can it be used to mean 'low virulence' or 'weak virulence' that is not, by all evidence thus far, transmissible?

K. W. Buck. Hypovirulence may be defined as the reduced ability to cause disease on plant hosts that lack any specific resistance genes to the pathogen. Because it describes a phenotype, the term can be used irrespective of whether the genetic determinant is cytoplasmically transmissible or non-transmissible. In describing individual hypovirulent isolates, reference should be made to the transmissible or non-transmissible nature of the genetic determinant when this is known.

J. W. Deacon (*Department of Microbiology, Edinburgh University, U.K.*). Would Professor Buck comment on recent reports that transmissible hypovirulence in a few pathogens such as *Rhizoctonia solani* is associated with plasmid DNA? Is it possible that this has been overlooked in a wider range of pathogens because the separation procedures for extracting dsRNA have destroyed DNA?

K. W. Buck. It has not been shown unequivocally that the hypovirulence determinant in the isolates of *R. solani*, which were reported to contain linear dsDNA plasmids, is cytoplasmically transmissible. It is also noteworthy that the sizes of the linear dsDNA plasmids are the same

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as those of linear dsRNA molecules found in other isolates of *R. solani*. Because dsRNA survives the method used to isolate these plasmids, in my view further characterization of the plasmids, e.g. by restriction endonuclease mapping, would be desirable to prove conclusively that they are not dsRNA.

It is likely that plasmid DNA (if present) would be overlooked when extracting dsRNA by the most commonly used procedure, i.e. cellulose chromatography. If the method included DNase treatment then plasmid DNA would be destroyed along with chromosome DNA.

R. R. M. Paterson (*C.A.B. International Mycological Institute, Kew, U.K.*). Approximately what percentage of those fungi tested have dsRNA viruses and what is the nature of the other viruses?

K. W. Buck. About 20%. However, only limited surveys have been done. I suspect the true figure to be higher.

Isometric single-stranded RNA and double-stranded DNA viruses have been described. Other morphological types of particle detected by electron microscopy, but not isolated, include rigid rods, filamentous rods, bacilliform particles, herpes-like particles, geminate particles and pleomorphic particles with membrane envelopes. Recently, retrovirus-like particles have been isolated from *Saccharomyces cerevisiae* and have been shown to be intermediates of DNA transposition.

C. Prior (*C.A.B. International Institute of Biological Control, Ascot, U.K.*). How host-specific are the viral pathogens of fungi?

K. W. Buck. The only known method of viral transmission between different fungal strains is by hyphal anastomosis, which would be expected to contain viruses within species. However, although related viruses are generally found in the same or closely related species, there are some interesting exceptions, e.g. related viruses have been found in *Penicillium stoloniferum* and *Diplicarpon rosae*. Methods that have been developed recently for infecting fungal protoplasts with isolated viruses should allow experimental host ranges to be determined.

G. Défago (*Swiss Federal Institute of Technology, Zürich, Switzerland*). What is known about the molecular basis of hypovirulence of *Endothia parasitica*?

K. W. Buck. Virulent strains of *E. parasitica* produce oxalic acid, which may weaken host tissue by combining with calcium, and which lower the pH, thus providing favourable conditions for the action of degrading enzymes such as polygalacturonase. Hypovirulent strains are deficient in oxalic acid production. However, there must be other factors contributing to hypovirulence. N. K. Van Alfen is currently comparing cDNA libraries of virulent and hypovirulent strains, which are isogenic in their nuclear genomes, and this work should eventually contribute to a more complete understanding of the molecular basis of hypovirulence.

C. C. Payne (*Institute of Horticultural Research, Littlehampton, U.K.*). Professor Buck implied that the dsDNA found in vesicles in hypovirulent *Endothia parasitica* could arise through the replication of an ssRNA virus. Is there any evidence that such a virus is related to RNA viruses of higher plants?

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K. W. Buck. The comparison has been made with the animal virus poliovirus on the basis of a blocked 5′ terminus and a polyA tract at the 3′ terminus. There are several groups of plant viruses with these structural properties; in terms of size, the potyvirus (potato virus Y) group is the most similar. We must await sequence analysis of *E. parasitica* dsRNA to see if these rather superficial similarities are upheld at the level of genome organization.

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