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Virus-like particles associated with dieback symptoms in the brown alga *Ecklonia radiata*

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ABSTRACT: Since 1991 epidemic dieback of the kelp *Ecklonia radiata* has been observed at the Leigh Marine Reserve and other sites in northern New Zealand. As the dominant phaeophycean *E. radiata* is a very important component of the eastern coastal ecosystem, providing both food and refuge for many species. Examination of the *E. radiata* sap extracts by electron microscopy detected the presence of both straight (ca 280 nm) and flexuous (ca 700 to 900 nm) virus-like filamentous particles. Extracts from several dieback-affected plants also reacted positively with potyvirus group monoclonal antibody.

KEY WORDS: *Ecklonia radiata* · Kelp · Virus-like particles · Potyvirus

INTRODUCTION

Virus-like particles have been detected in at least 44 taxa of eukaryotic algae (Van Etten et al. 1991). In most cases reports are of a single observation of particles during microscopic studies and characterisation of these particles has not been pursued. Polyhedral virus-like particles have been detected in the green algae, red algae and filamentous brown algae (Lee 1971, Markey 1974, Dodds 1979, Van Etten et al. 1991, Müller & Stache 1992), including 2 species of filamentous brown algae from New Zealand (Henry & Meints 1992, Müller & Stache 1992). All of these viruses exhibit similar structural characteristics, being polyhedral particles with a double stranded DNA genome (Müller & Frenzer 1993, Friess-Klebl et al. 1994).

Chara australis virus (Gibbs et al. 1975), a virus infecting the freshwater macrophytic green alga *C. australis*, is the only known algal virus which resembles vascular plant viruses (Van Etten et al. 1991). This virus has been isolated from the sap of *C. australis* and apart from its size (530 nm long) resembles the particles of tobacco mosaic virus (Gibbs et al. 1975, Skotnicki et al. 1976). Infection of healthy cells by injection with purified particles results in chlorosis and death in 10 to 12 d (Skotnicki et al. 1976) and replication of virus particles is also seen to occur within infected cells (Gibbs et al. 1975.) The genome of this virus has been characterised and consists of a single-stranded RNA. The nucleotide sequence (Brunt & Richards 1989) suggests that it may be more closely related to the furoviruses than the tobamoviruses (Van Etten et al. 1991).

Noticeably absent from the types of algae from which viruses have been recovered are the larger fucoid and laminarian algae. This is possibly because most viruses or virus-like particles found in the algae have been discovered by chance during ultrastructure studies related to the life history, rather than during investigation of disease.

Ecklonia radiata (C. Agardh) J. Agardh 1848, is a large perennial kelp (Laminariales, Alariaceae) which is common along the eastern coastline of New Zealand, where it is the dominant phaeophycean (Novaczek 1980) and forms extensive forests. In late 1991 dieback of the kelp was observed at about 15 m depth in a restricted area of the Cape Rodney to Okakari Point Marine Reserve in Northern New Zealand. Affected plants are characterised in the early stages by light-coloured patches on the laminae, associated with increased rates of erosion of laminae. The number and size of the laminae are reduced until only the stipe remains, before eventual disintegration, when even the holdfast disappears (Babcock & Cole 1993, Cole & Babcock 1996) (Fig. 1).

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Fig. 1. *Ecklonia radiata*. Progressive symptoms of dieback. (A) Unaffected, (B) early stage lesion showing reduction in pigmentation, (C) advanced degradation, (D) frond showing various stages of dieback symptoms

Extensive dieback in other parts of the reserve was noticed in November 1992, with large numbers of plants at 15 to 20 m depths being affected. Between January and May 1993 plants in mid-depths (9 to 12 m) became increasingly affected, while deeper plants (15 to 18 m) in some areas suffered 90 to 100% mortality. Throughout 1993 the dieback continued spreading to shallow depths which were initially unaffected (Babcock & Cole 1993). Similar dieback has also been reported at a number of other sites in northern New Zealand, but none so extensive, or as well documented as that observed in the Cape Rodney to Okakari Point Marine Reserve (Babcock & Cole 1993, Cole & Babcock 1996).

MATERIALS AND METHODS

Electron microscopy. Small pieces (3 to 4 mm) of dieback-affected kelp tissue were crushed in 1% aqueous phosphotungstic acid, pH 7.0 and a drop of the stain/sap mixture placed onto a parlodion-coated, carbon-stabilised copper grid. Excess liquid was removed and the negatively stained samples viewed under a Philips 310S transmission electron microscope. Sap extracts from healthy kelp were used as negative controls.

Plant inoculations. Extracts of kelp laminar tissue from plants at 5 different stages in symptom development were mechanically inoculated to *Chenopodium quinoa*, *Petunia hybrida*, *Nicotiana benthamiana*, *N. glutinosa*, *N. tabaccum*. The kelp laminar tissue was triturated in 0.01 M Sorensens phosphate buffer, pH 7.2 (1:10, w/v) using a pestle and mortar and rubbed gently on expanded leaves of the test plants which had been dusted lightly with 500 mesh carborundum.

Serology. Plant samples were tested for the presence of potyvirus using a potyvirus virus group monoclonal antibody (Agdia Corp., Elkhart, USA) in an antigen coated plate (indirect) ELISA, according to the suppliers protocol. Tissue samples from kelp and herbaceous species were homogenised 1:10 w/v and 1:100 w/v, respectively, in the extraction buffer (sodium carbonate/bicarbonate, pH 9.6). These ratios were chosen following optimisation experiments (data not shown). Following the addition of the enzyme substrate, the Abs_{405} (absorbance at 405 nm) was measured after 30 min incubation in the dark using a Bio-Tek EL307B EIA reader. Healthy *Ecklonia radiata* plants from a site where dieback is absent were used as controls. Samples were tested in triplicate and were regarded as positive when Abs_{405} readings were greater than the mean of the control plus 3 standard deviations. Sap from dasheen mosaic potyvirus (DMV)-infected taro was used as a positive control.

RESULTS

Electron microscopy

Two types of virus-like particles (VLPs) were observed in lamina samples of diseased *Ecklonia radiata*, rod-shaped (tobamovirus-like) particles 280 nm long (Fig. 2a) and flexuous filamentous (potyvirus-like) particles 700 to 900 nm long (Fig. 2b). Results are summarised in Table 1. VLPs were observed in 7 of the 15 diseased plants examined, although they were not observed in all of the samples taken from each plant. Tobamovirus-like particles were observed in 4 of the plants, while potyvirus-like particles were observed in 5. On 2 occasions both types of particles were observed in samples from the same plant. The numbers of particles observed on each grid was very low, with only 9 or 10 particles seen on the best grids. No VLPs were observed in extracts of healthy kelp, or in stained phosphate-buffered saline (PBS) controls.

Plant inoculations

No local lesions or systemic virus-like symptoms developed on any of the herbaceous plants inoculated with sap extracts from dieback-affected *Ecklonia radiata*. Enzyme-linked immunosorbent assay (ELISA) tests on the inoculated plants 4 wk after inoculation using the potyvirus group antibody were all negative.

Serology

Five out of 15 dieback-affected *Ecklonia radiata* plants gave clear positive reactions to the potyvirus group test when mature laminar was tested (Table 2). In addition, 2 apparently healthy plants collected from a dieback site gave a positive potyvirus test. These

Table 1. *Ecklonia radiata*. Presence of virus-like particles in negatively stained sap samples from dieback-affected and healthy plants

Sample type	Number examined	Number of samples containing virus		
		Flexuous and straight rods	Flexuous rods only	Straight rods only
Dieback-affected	15	2	4	3
Healthy	6	0	0	0
Buffer control	5	0	0	0

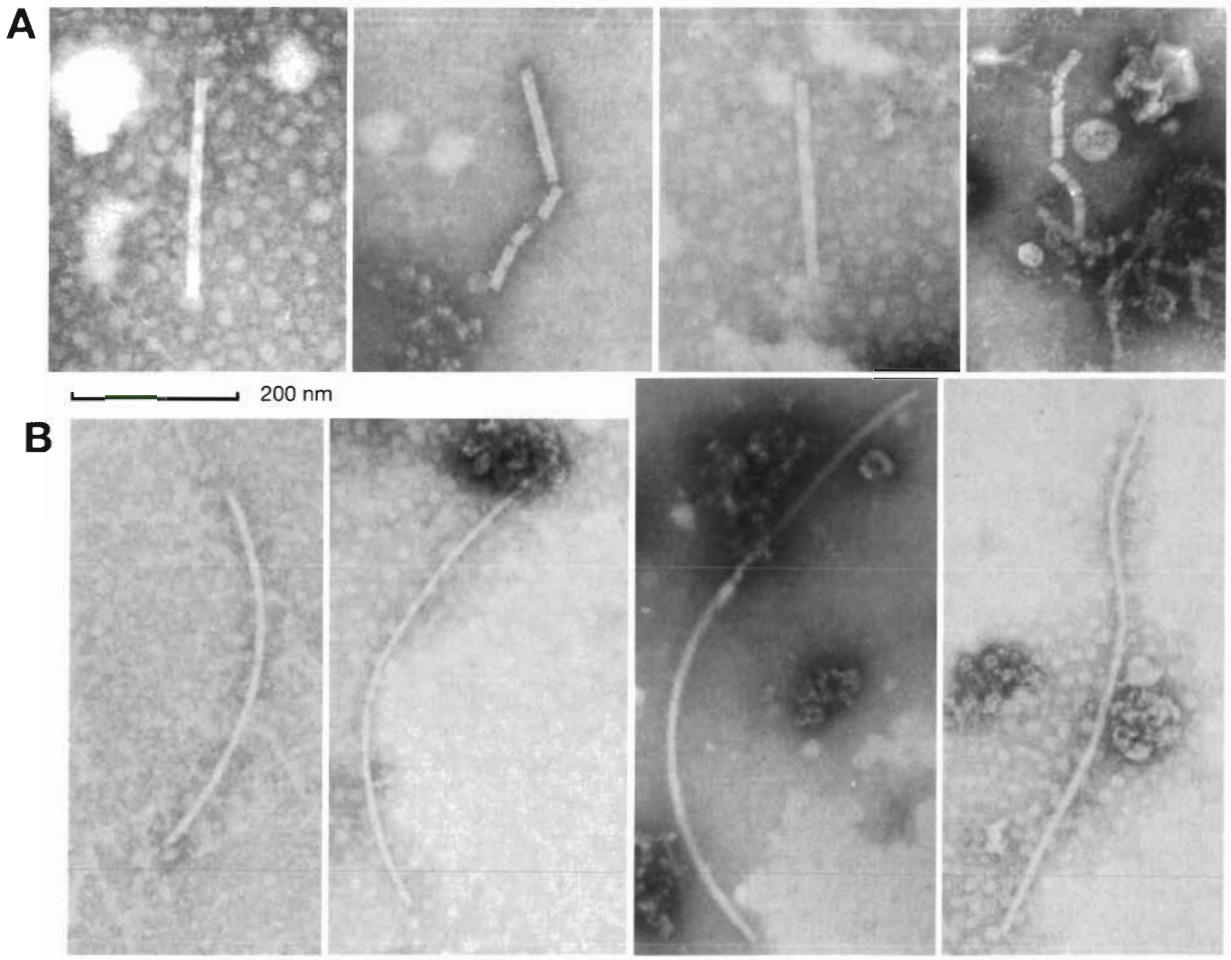

Fig. 2. *Ecklonia radiata*. Electron micrograph of virus-like particles observed in the alga. (A) Tobamovirus-like particle(s), (B) potyvirus-like particles

plants subsequently developed progressive dieback symptoms with the associated increase in ELISA Abs_{450} readings (Fig. 3). A subsequent comparison of potyvirus detection in different tissues of dieback-affected plants indicated that virus was most likely to be detected in mature laminar and sorus (Table 2).

DISCUSSION

Two types of virus-like particles were observed only in diseased *Ecklonia radiata*. The smaller rigid straight rods were similar in size and appearance to tobamoviruses (300 nm long) and furoviruses (250 to 300 nm) (Francki et al. 1991). The flexuous filamentous particles were similar in appearance to members of the potyvirus group, which range in length from 680 to 900 nm (Francki et al. 1991). The potyvirus-like particles were more frequently associated with dieback symptoms than were the tobamovirus-like particles.

Although VLPs were observed in only 7 of 15 diseased samples examined by electron microscopy, they

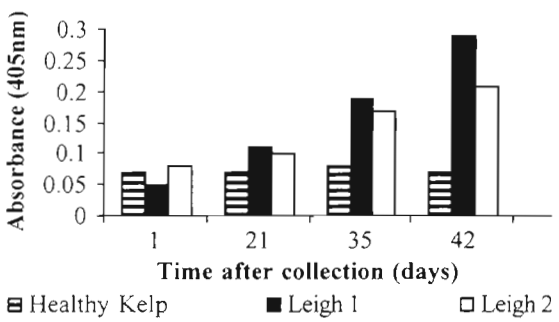

Fig. 3. *Ecklonia radiata*. Change in potyvirus detection by ELISA in 2 plants developing dieback symptoms in laboratory culture as compared to healthy controls

Table 2. *Ecklonia radiata*. Presence of potyvirus in plants as determined by ELISA using the Agdia™ potyvirus group test

Kelp sample type	Plants tested[a] (no.)	Clear positive[b]	Marginal positive[c]	Negative
Healthy (mixed tissue types)	14	0	0	14
Dieback-affected				
Batch 1				
Mature laminar	25	14	3	8
Batch 2				
Sorus	15	5	1	9
Young secondary laminar	2	0	0	2
Mature secondary laminar	9	5	1	3
Primary laminar	3	0	0	3
Meristem	3	0	0	3
Stipe	3	0	0	3

[a]Results are based on the mean of 3 replicate samples from each plant tested
[b]A plant was considered a clear positive when the mean absorbance at 405 nm (Abs_{405}) for the 3 replicate samples was greater than the mean Abs_{405} plus 3 standard deviations of the controls
[c]A plant was considered a marginal positive when the mean Abs_{405} for the 3 replicate samples was greater than the mean Abs_{405} plus 2 standard deviations of the controls (but less than the mean plus 3 standard deviations)

may also have been present in some of the other samples. It is not unusual to obtain negative results by electron microscopy, especially when as in this case, particles are in very low concentration. Nicolaieff & van Regenmortel (1980) showed that the adsorption of virions to grids is a highly selective process and that the particles which remain on the grid are not a random sample of those present in the suspension. Different particles possess variable specific affinities for the film surface, and, in addition, a 'wash-off' phenomenon often occurs when particles are stained (Milne 1984). Crude sap constituents have also been found to affect the adsorption of virus particles to grids (Nicolaieff & van Regenmortel 1980, Milne & Lesemann 1984), and the viscous nature of kelp extracts could have an inhibitory effect. All samples were highly viscous, and large amounts of cellular debris were observed on grids. Microfuged samples had lesser quantities of cellular debris present; however viscosity of the extracts was still considerable. It is possible that treatment of extracts prior to adsorption onto electron microscope grids could result in observation of greater numbers of virus particles. However, some viruses occur in such low concentration that detecting them by simple electron microscope techniques is unreliable (Hill 1984). It is possible that the VLPs present in *Ecklonia radiata* dieback affected plants may fall into this category. In addition, for many host/virus combinations the likelihood of finding particles in an electron microscope preparation varies with the age of the plant and plant part chosen for study (Hill 1984), and consistent virus recovery generally requires some knowledge of the host/virus relationship. Preliminary results for different tissues indicate that there are tissue differences which should be further studied. As this knowledge of kelp-associated viruses is not available, it is not unexpected that viruses were not detected in all samples.

The serological tests indicated the presence of a potyvirus (or serologically related virus) in some diseased *Ecklonia radiata*, although a consistent association with dieback symptoms was not observed. Typically the Abs_{405} for a positive sample was only 3 times greater than that for healthy samples. This may be due in part to low virus titre but it was also observed that the addition of 10% healthy kelp extracts to DMV-infected taro extracts depressed the Abs_{405} readings by 60%. Since taro sap itself depresses the Abs_{405} of purified DMV solutions (Saelea 1996), this suggested that kelp extract interferes with the potyvirus group tests to a sustantial degree.

The ELISA data (Table 2) also indicate that the results are influenced by the type of tissue tested with mature laminar giving the greatest number of positive results. The stage in disease development may also be a factor.

This is the first reported case of VLPs found in a laminarian kelp. The absence of other reports of viruses in kelps is, most likely, due to lack of research in this field, rather than reports that the virus is absent (Dodds 1979, Van Etten et al. 1991). Previous virus and VLP discoveries in algae have occurred during microscopic investigations of the ultrastructural aspects of algae life history, rather than an investigation of a disease (Van Etten et al. 1991). The observed presence of 2 different types of VLPs indicates that VLPs may in fact be relatively common in kelps. This has been found to be the case for filamentous brown algae (Müller & Stache 1992). It is possible, and relatively common in nature, for an individual plant to be simultaneously infected with 2 or more distinct viruses, which may interact imperceptibly, antagonistically, or synergistically (Cooper & MacCallum 1984). While the majority of dieback-affected plants (68%) tested positive to the Potyvirus Group antiserum, this does not rule out the observed tobamovirus-like particle as a cause of the dieback. It is possible, based on morphology, that this particle may in fact be related to the furovirus group, as is the only other rod-like algal virus, *Chara australis* virus (CAV) (Van Etten et al. 1991).

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