Relationships Between the Pathogen *Oplidium virulentus* and Viruses Associated with Lettuce Big-Vein Disease

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Lettuce big-vein disease (LBVD) is a widespread problem in commercial lettuce producing areas. It was first reported in 1934 in California, USA (28). Much of the early foundation research on this disease was carried out by R. N. Campbell and associates at the University of California, Davis. In the United States in 2009, it was estimated that 100% of all commercial lettuce production was concentrated in California (in early spring) and Arizona (in winter) (68). The disease also occurs worldwide, particularly in regions in Australia, Europe, Brazil, and New Zealand, and at high altitudes in subtropical regions. LBVD often results in decreased yield of produce from infected plants, mainly in winter growing seasons. Symptoms of this disease are the obvious chlorophyll clearing (Fig. 1), which causes the big-vein leaf symptoms from which the disease gets its name, along with crinkled leaves and a reduced head size, which is often oblong in shape (Fig. 2).

LBVD is associated with a complex of two viruses, *Lettuce big-vein associated virus* (LBVaV; genus *Varicosavirus*) and *Mirafiori lettuce big-vein virus* (MLBVV; genus *Ophiovirus*) (51). Motile zoospores of *Oplidium virulentus* vector LBVaV and MLBVV to the roots of healthy plants and, in the absence of susceptible hosts, its resting spores retain the ability to harbor these viruses for decades in infested soil (2,5,38,51) and in dry roots for 39 months (6). The ability of viruliferous resting spores to persist in production areas for such extended periods of time, and also the motility of viruliferous zoospores, pose significant difficulties for the management and/or control of the disease. The severity of LBVD is amplified given that lettuce production systems are often intensive, irrigated, and on land that is already infested with LBVaV and MLBVV.

Oplidium spp. are considered to be the closest living flagellated organisms related to terrestrial fungi (59). As a member of Chytridiomycota (chytrids), they have previously been classified within kingdom Protista; however, they are now regularly classified within kingdom Fungi. *Oplidium* spp. are cosmopolitan in distribution, and the species vectoring the viruses associated with LBVD (*O. virulentus*) has been found to be an ubiquitous obligate root-infecting fungal parasite (22).

Previously, it was accepted that LBVaV was the virus responsible for LBVD symptoms; however, Lot et al. (38) and Sasaya et al. (57) described MLBVV as being the causal agent of big-vein symptoms of lettuce. Also, until recent molecular studies of the chytrid vector, it has been thought that the vector for both LBVaV and MLBVV was *O. brassicae*, when in fact it was determined to be a different species, *O. virulentus* (33,40,60).

Oplidium spp.

Oplidium spp., especially *O. virulentus*, are rarely reported to be seriously pathogenic, although their life in roots is clearly parasitic. There is no published record of deleterious effects of host infection by *O. virulentus*; however, *O. bornovanus* causes significant necrosis of melon roots (62) resulting in reduced shoot and root growth and therefore reduced market value.

O. brassicae was first described in 1939 (55) and then first associated with big-vein disease of lettuce by Fry in 1958 (19). It was in 1961 that graft transmission of big-vein disease in lettuce was first reported and the suggestion was made that the disease was caused by a virus (10). Campbell and Grogan in 1963 (7) proposed *O. brassicae* as the vector of the virus that caused big-vein disease in lettuce. However, Sahtiyanci (54) proposed that, based on differing hosts ranges, there were in fact two separate species of *Oplidium* responsible for virus transmission, a crucifer-infecting strain, *O. brassicae*, and a non-crucifer-infecting strain, *O. virulentus*. In that same study, Sahtiyanci (54) also found that *O. brassicae* was heterothallic, whereas *O. virulentus* was homothallic. Sahtiyanci (54) also proposed that due to *O. brassicae*, *O. virulentus*, and *O. bornovanus* sporangia having more than one exit tube, they should all be reassigned to the genus *Pleotrachelus*. Campbell and Lin (11) disputed the work of Sahtiyanci (54) on the basis that they found *O. brassicae* sporangia to have single exit tubes and hence should remain in the genus *Oplidium*. Campbell and Lin (11) also disputed the separation of two strains of *O. brassicae*, because even though they had different host ranges, they were unable to infect plants with single-sporangial isolates of each strain. Campbell and Lin (11) did, however, also find *O. virulentus* to be homothallic, but they could not determine the nature of *O. brassicae* in this regard and concluded that there was not enough specialization to separate *O. virulentus* from *O. brassicae*. As a consequence, *O. brassicae* has continued to traditionally be regarded as the vector of LBVaV and MLBVV.

The development and implementation of molecular techniques decades later allowed Sasaya and Koganezawa (58) to determine that *O. brassicae* and *O. virulentus* are in fact different species and not strains of the same taxon. They also determined that *O. virulentus*

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tus, not *O. brassicae*, is the vector of MLBVV and that both *Olpidium* spp. have different host ranges, with *O. brassicae* commonly infecting crucifers (cabbage and mustard) and *O. virulentus* commonly infecting noncrucifers (lettuce and tomato), supporting the earlier findings of Sahtiyanci (54). It should be noted that in literature prior to Sasaya and Koganezawa (58), references to *O. brassicae* may not always relate to this taxon and may have in most cases erroneously dealt with *O. virulentus* instead. It is interesting that in the study in southwest Australia by Maccarone et al. (40) testing for both LBVaV and MLBVV, *O. brassicae* was detected in only one root extract out of 80 tested. This study was the first report of LBVaV being associated with *O. virulentus*, as previous work done by Sasaya and Koganezawa (58) investigated MLBVV and *Tobacco stunt virus* (TStV), but not LBVaV.

Phylogenetic analysis of ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences of five southwest Australian isolates of *O. virulentus* showed that these isolates belonged to two subclades, suggesting two introductions of *O. virulentus* into the region (40). The first subclade included four of the five isolates as well as isolates from Europe and Japan, while the second subclade included one isolate from Japan. It is interesting to note that both southwest Australian and Japanese isolates are represented in both subclades, suggestive that both may have come from the same source. Another possible scenario is that they occur concurrently around the Western Pacific Rim. This could be established only if a wider study is carried out of the populations that occur in other countries in this region where lettuce is grown.

***Olpidium* – Plant Associations**

Susceptibility of host plant taxa to *O. virulentus* is essentially based on the amount of sporangia produced in host roots. As an

invader of fine roots, the fungus employs a rapid invasion pattern to suit its short and simple life cycle. For example, the work of Campbell et al. (9) found that, following inoculation, *Olpidium* produced mature sporangia in 3 to 4 days with the symptoms of LBVD appearing in 3 to 4 weeks. Differences in vegetative generations may vary with species, *O. virulentus* taking 3 days as compared to *O. bornovanus*, which requires 5 days to complete a generation (9).

In early studies prior to the molecular characterization of *O. virulentus*, it was assumed that *O. brassicae* was the vector of the viruses associated with LBVD in lettuce. Considering recent work, especially from Spain (25) and southwest Australia (40), it is clear that *O. brassicae* is not common in horticultural fields, and even where it did occur (in <5% of the plants studied), it occurred only on broccoli and cabbage (family Cruciferae) (25). In the United Kingdom, Hartwright et al. (22) described three different *Olpidium* isolates based on phylogenetic analysis of the rDNA ITS region sequence and host range. *O. brassicae* was found to infect broccoli but not lettuce or carrot, while one isolate failed to infect cucumber. *Olpidium* isolated from lettuce failed to infect broccoli or carrot, and one isolate did not infect cucumber. *Olpidium* isolated from carrot infected lettuce but failed to infect broccoli or cucumber. From this study, Hartwright et al. (22) proposed that broccoli-infecting isolates still be referred to as *O. brassicae*, while lettuce-infecting isolates be referred to as *O. compositae* because they infect other members of the family Compositae, and that the third group of isolates found to infect cucumber be referred to as *O. cucurbitaceae*.

O. bornovanus was observed on 53% of all the plants studied by Herrera-Vasquez et al. (25), which were collected over a 10-year period from Brazil, Guatemala, Honduras, Mexico, Panama, Portu-

Fig. 1. A commercially grown lettuce plant from southwest Australia exhibiting symptoms of lettuce big-vein disease, specifically chlorophyll clearing of the leaves.

gal, Spain, the Netherlands, Tunisia, Uruguay, and the United States. It was clearly the most common of the three *Olpidium* species they studied. Although more *Olpidium* species are described in the literature, the scientific verification of these species is still to be carried out because most of them have not yet been identified using molecular methods.

O. virulentus has been shown, using molecular diagnostic techniques, to be the vector for LBVD in Japan and southwest Australia (40,58). Under natural conditions, *O. virulentus* has been reported in Europe (22) and South America (26) on melon, watermelon, and tomato in addition to lettuce.

Olpidium – Virus Associations

Fungal viruses are common in nature, and *Olpidium* spp. transmit at least 18 viruses to many different host plant species (Table 1). They persistently infect fungal hosts, often in the absence of apparent symptoms, to the extent that Dawe and Nuss (15) considered these viruses to be intrinsic fungal genetic elements. As

asymptomatic mycoviruses, they may impact fungal ecology and evolution. For example, hypovirulence of the chestnut blight fungus (*Cryphonectria parasitica*) is associated with members of the virus family Hypoviridae (15). Similar reduction in *Rhizoctonia* fungi has been related to the presence of double-stranded RNA (dsRNA) viruses (13). Many fungal viruses are comprised of dsRNA, so its presence within fungal cells can signal virus infection (73). Complex evolutionary benefits from associations of fungi and viruses may occur more commonly than currently acknowledged. An excellent example is the three-way symbiosis required for the thermal tolerance of tropical panic grass which is conferred by the fungus *Curvularia protuberata* only when the endophyte is carrying *Curvularia thermal tolerance virus* (CThTV) (43). Fortunately for *Olpidium*, there appears to be no trade-off by the fungal host during the parasitic phase of the virus in the fungus.

Campbell and Grogan (8) and Lot et al. (38) assumed that the viruses associated with LBVD are carried internally (in vivo) in the

Fig. 2. Lettuce plants grown on a commercial lettuce growing property in southwest Australia showing symptoms of lettuce big-vein disease (LBVD). The lettuce plant on the left does not show any visible LBVD symptoms, while the plant in the center has severe symptoms such as leaf crinkling that prevented head formation. The plant on the right shows the oblong-shaped head associated with LBVD.

Table 1. *Olpidium* spp., viruses they vector, and host plants commonly infected by the virus, expanded from Rochon (50)

<i>Olpidium</i> species	Virus transmitted	Acronym	Host plant
<i>O. bornovanus</i>	<i>Cucumber necrosis virus</i>	CNV	Cucumber
	<i>Cucumber leaf spot virus</i>	CLSV	Cucumber
	<i>Cucumber soil-borne virus</i>	CSBV	Cucumber
	<i>Melon necrotic spot virus</i>	MNSV	Melon
	<i>Squash necrosis virus</i>	SqNV	Squash
	<i>Red clover necrotic mosaic virus</i>	RCNMV	Red clover
<i>O. brassicae</i>	<i>Chenopodium necrosis virus</i>	ChNV	Chenopodium
	<i>Lisianthus necrosis virus</i>	LNV	Lisianthus
	<i>Tobacco necrosis virus-A</i>	TNV-A	Tobacco
	<i>Tobacco necrosis virus-D</i>	TNV-D	Tobacco
	<i>Tulip mild mottle mosaic virus</i>	TMMMV	Tulip
	<i>Freesia sneak virus</i>	FreSV	Freesia
	<i>Lettuce ring necrosis virus</i>	LRNV	Lettuce
	<i>Olive mild mosaic virus</i>	OMMV	Olive
	<i>Mirafiori lettuce big-vein virus</i>	MLBVV	Lettuce, <i>Sonchus hydrophyllus</i> ^a
<i>O. virulentus</i>	<i>Lettuce big vein associated virus</i>	LBVaV	Lettuce, <i>S. hydrophyllus</i> [*]
	<i>Pepino mosaic virus</i>	PepMV	Tomato
	<i>Tobacco stunt virus</i>	TStV	Tobacco

^a * = Native plant species.

zoospores and resting sporangia of *O. brassicae*. Campbell and Grogan (8) found that washing zoospores and altering the pH of the zoospore suspension did not result in reduced incidence of virus transmission. In vivo acquisition is also supported given that resting spores can remain viruliferous in soil for decades. Other viruses such as *Tobacco necrosis virus* (TNV) are not found inside resting spores but adsorb to the surface (in vitro) of *O. brassicae* zoospores (64). Therefore, in contrast to viruliferous *O. virulentus* resting spores, the survival and spread of externally carried viruses is dependent upon virion stability within the soil and crop debris postharvest. This represents significant differences in disease management strategies, as crop rotation with nonhost plants can be used to eliminate viruses not contained within vector resting spores.

There are clear indications of specialization in relation to the recognition of the viruses to their fungal hosts. For example, studies by Kakani et al. (30) established that the binding of the *Cucumber necrosis virus* (CNV) to the surface of vector zoospores (*O. bornovanus*) involves recognition of the fungal oligosaccharides. All zoospores of *O. bornovanus* are virus free at discharge. The vector zoospores and the CNV particles are so intimately associated that it takes just 5 to 10 min for the zoospores to become infected after discharge (18).

Also, it has recently been shown that virus particles of mutant strains of *Olive mild mosaic virus* (OMMV) that contain two amino acid changes on the coat protein attach to *O. brassicae* zoospores less efficiently than wild-type virus (70). Early studies of Stobbs et al. (63) showed that the entry of the virus into the *O. bornovanus* zoospores involved adhesion of CNV to the plasmalemma and the axonemal sheath of the zoospore. The virus entered the cytoplasm through the “reeling-in” method of flagellar retraction and endocytosis. There was no evidence of mechanical “pushing” of the virus into the zoospores.

While the general aspects of virus acquisition are understood, the mechanism by which LBVaV and MLBVV are acquired and released into plant hosts by *Olpidium* is unknown. Further investigation using labeled microscopic techniques would give visual validation of the presence of LBVaV and MLBVV virus particles inside *Olpidium* zoospores and resting spores. Additionally, targeted molecular investigation of virus coat proteins and zoospore plasmalemma interactions would prove valuable in determining mechanisms of virus transmission.

Lettuce – Virus Relationships

The diagnosis of MLBVV associated with LBVD in southwest Australia was not unexpected as it was established as the causal agent of LBVD by previous work done in Europe (45,47,69), Japan (32), and America (24,56) where the disease causes similar symptoms to those observed in southwest Australia. It is interesting that in the study by Maccarone (39) of commercial lettuce fields, lettuce, *Sonchus oleraceus*, and one plant of *Arctotheca calendula* [family Asteraceae] were the only other species among those tested found naturally infected with MLBVV in the field. This indicates that the natural host range of LBVaV and MLBVV may be limited, although the disease is widespread as suggested previously (11,22,54). Therefore, LBVaV and MLBVV successfully survive and spread in the southwest Australian environment (35,36).

Phylogenetic examination of coat protein (CP) sequences of MLBVV reveal that isolates group into two distinct clades, A and B, with clade A further divided into subclades A1-A3 (41). Navarro et al. (46) previously reported the existence of two clades, but did not recognize subclades as determined when isolates from southwest Australia were included in the phylogeny (41). Of 13 CP isolates sequenced from southwest Australia, 12 were included in subclade A1, while one isolate fit into subclade A3, which also included isolates from Europe and Japan (41). This indicates that there were possibly at least two introductions of MLBVV into southwest Australia. Clade B contained isolates of MLBVV only from Spain, reflecting the Mediterranean region as the center of domestication of the crop (72). Lettuce is thought to have origi-

nated in Egypt and then was cultivated in the southern regions of the Mediterranean basin (37). Thus, it is likely that the virus and the host were spread together, and the presence of two clades which appear to show close identity indicates that they were separated by an evolutionary event.

The high sequence identities shared by CPs of the 12 Australian MLBVV isolates in subclade A1 is evidence that the fungal vector and these virus isolates may have originated from a single introduction. The presence of the isolate in subclade A3 indicates a second introduction of the virus. Of interest would be a comparison of MLBVV isolates from other horticultural areas within Western Australia, South Australia, Victoria, Queensland, and Tasmania where lettuce is commercially grown to determine incidence and distribution of members of both subclades, and to establish if members of clade B occur widely on the Australian continent.

Maccarone (39) also reported, for the first time, infection of two Australian native plant species (*Actites* sp. and *S. hydrophilus*) by MLBVV. It is not known how many other native Australian plants are symptomatic or asymptomatic carriers of MLBVV. Such knowledge is important because southwest Australia experiences a Mediterranean climate where in summer all annual plants die unless they are irrigated. The perpetuation of the virus in annual plants such as *Reichardia* sp., *S. asper*, *S. oleraceus*, and *S. hydrophilus* could be limited to the presence of viruliferous *O. virulentus* resting spores, although this may not account for the widespread occurrence of this virus. Even if there was only limited carryover of the virus, the fact that Australian native plants can be reservoirs of MLBVV could be significant for both Australian flora and commercial lettuce production. For example, the native West Australian plant *Kennedia prostrata* has been shown to be a disease reservoir of the exotic virus *Bean yellow mosaic virus* (BYMV), causing symptoms such as generalized leaf distortion, vein clearing, chlorotic mosaic, and severe plant dwarfing (71).

The exact role of LBVaV in LBVD still remains unclear, and it is generally considered that infection with this virus does not induce symptoms in lettuce (14,52). It is also not known whether this virus has an additive or synergistic effect on the expression of the disease in the presence of MLBVV. However, while Colariccio et al. (14) and Roggero et al. (52) both report no evidence of synergism, Roggero et al. (52) suggest this lack of synergism may reflect the poor detection of the two viruses rather than proof of LBVaV causing symptoms or adding to the level of symptom expression when both viruses are present. In tests in which LBVaV and MLBVV were baited out of infested soil using seedlings, 88% of the soils assayed by Maccarone (39) from lettuce production nurseries were positive for MLBVV, and 69% of the samples were positive for both LBVaV and MLBVV, suggesting lettuce is susceptible to coinfection by both these viruses even though there is little indication that the two viruses are interacting with each other. Given the suspected latency of some strains of LBVaV, it is possible that it is an ancient virus partner of wild lettuce and therefore does not express symptoms (53).

An analysis of CP sequences of seven LBVaV isolates from southwest Australia and those available on GenBank reveal the existence of two clades (42). All isolates from southwest Australia were more closely related to the Japanese isolates of LBVaV and TStV (clade II) than to LBVaV isolates from Europe (clade I). This study also showed that the CP of LBVaV is even more highly conserved than that of MLBVV isolates. The geographically distinct clades and lack of genetic diversity among Australian isolates may reflect founder effects and therefore its recent introduction. For example, Tsompana et al. (66) attributed the geographic structure of *Tomato spotted wilt virus* (TSWV) to founder effects. Conversely, *Lettuce necrotic yellows virus* (LNYV), which was first described in Australia, has been postulated as originating from plant species endemic to Australia (49). It is most likely that the highly conserved nature of the LBVaV CP coding region is the result of recent introduction, and evolutionary processes are yet to have occurred. This may be further explained by the fact that Australia has a relatively recent cultivation history (~200 years), and it

is speculated that LBVD was introduced during this time via the import of infested soil and plant material.

Disease Management Strategies

Given the thick cell-walled nature of *O. virulentus* resting spores and their persistence in the soil for greater than 20 years, their control is most challenging. Previously, the use of the volatile chemical methyl bromide has been used to control *O. virulentus* resting spores. However, methyl bromide was recognized as an ozone depleting substance and has been banned in the United States and in 159 other countries worldwide who signed "The Montreal Protocol on Substances that Deplete the Ozone Layer" in 1997 for a complete phase-out of methyl bromide for nonquarantine purposes by 2005. Therefore, more environmentally sensitive control methods are sought in order to reduce the spread and severity of LBVD.

Although there are clear indications of host response to both vector and viruses, much more research needs to be done on durable resistance to the disease. Few options for agronomic host resistance are available. However, partially resistant lettuce cultivars have been achieved by breeding commercial lettuce cultivars with *Lactuca virosa* (wild lettuce), which does not develop LBVD symptoms (3). Hayes and Ryder (23) had success in developing partial resistance when *L. virosa* alleles were introgressed into *L. sativa*. Kawazu et al. (31) used a different approach and transformed LBVaV coat protein genes into lettuce, resulting in transgenic lettuce that showed resistance to LBVD. The authors made the suggestion that this method could be used to develop resistant cultivars and/or breeding stock.

However, given the nature of the chytrid, realistically, management strategies for LBVD highlighted here focus on prevention of disease spread and introduction of new strains rather than disease elimination (control). This is particularly relevant to virgin farming land, which is assumed to currently be free of the disease and the reduction of inoculum potential. Firstly, in order to employ better management strategies for LBVD, we must better understand the source(s) of inoculum to prevent the spread of the vector and/or the viruses from production nurseries to lettuce growers who have started cultivation on virgin lands which are assumed to be free of LBVD contamination. Secondly, a further reason why management of LBVD is functional is evident in the work done by Latham et al. (36) which shows that early infection of lettuce seedlings with LBVD cause more severe symptoms and greater yield loss than

infections which occur later once the seedlings have been planted in the field (Fig. 3). Therefore, prevention of LBVD at the early stages of lettuce production, for example in commercial seedling nurseries, may increase yields.

Disease Management – Commercial Lettuce Seedling Nurseries

Of importance to commercial lettuce seedling growers, Maccarone (39) found that the lettuce seeds can carry viruliferous *O. virulentus* resting spores externally on the seed coat. This is significant because even with nursery hygiene, potting mix sterilization, and all other sanitary precautions that should ideally be associated with lettuce seedling production in a seedling nursery, lettuce seeds still can bring the disease into the nursery (No. 1, Fig. 3). Surface sterilization of seed to eliminate viruliferous *O. virulentus* resting spores would be required to overcome this. This is a significant finding in relation to the epidemiology of LBVD. This is supported by studies in Japan by Faruki (17) and in the United States by Campbell et al. (12), who showed *O. bornovanus*, the vector of Melon necrotic spot virus (MNSV), to be externally seedborne. Therefore, in addition to the constituents of potting mix being a potential source of inoculum as reported by Latham et al. (36), seeds too should be considered as an alternative source of infection, which could be negated by the use of surface sterilization of the seeds prior to being coated. Most lettuce seeds are coated with an inert substance that makes the seeds all a uniform shape for ease of handling. This practice could inadvertently coat viruliferous *O. virulentus* resting spores onto the lettuce seed coat.

Hardy and Sivasithamparam (21) reported that Western Australian nurseries carry eight different species of *Phytophthora* and proposed that nurseries were the source of new strains or species of that pathogen in southwest Australia. Similarly, it is possible for production nurseries to supply lettuce seedlings grown from contaminated seeds that could introduce new strains and/or species of viruses into the farming environment. Although LBVaV and MLBVV have not been detected internally within lettuce seed (in the embryo), external transmission in the vector resting spores could be serious even at low concentrations because that can result in the introduction of LBVD to uninfested land and production areas. For example, there has been reported vector-dependent virus transmission in which *O. bornovanus* zoospores acquired and transmitted MNSV that contaminated the surface of melon seed (17). A similar seed/pathogen relationship is described with white

Fig. 3. Flow chart representing the possible sources of inoculum of lettuce big-vein disease (LBVD) carried in/on *Olpidium virulentus* both in the seedling nursery environment and also in the lettuce production fields in Western Australia. These potential sources as proposed by Maccarone (39) and Jones (29) are indicated by the broken arrows, while inoculum sources determined by Latham et al. (36) have been indicated by solid arrows.

rust on brassicas with Barbeti (1) reporting that the seeds of *Brassica rapa* carried oospores of *Albugo candida* (white rust) externally. These oospores could then function as the means of introducing the disease into commercial oilseed brassica fields in areas where the disease never existed before.

Weeds may play an important role in the spread and the carry-over of the vector and the virus despite the long-term survival of viruliferous resting spores in soil. *S. oleraceus* and *A. calendula* are common weed species in agricultural regions of southwest Australia. The assumption that these weeds may be implicated in the spread and survival of LBVD indicates that management of these weeds, even though it would not eradicate LBVD, would help to reduce both the level of inoculum potential in the soil over a period of years and subsequent incidence/severity of the disease (No. 9, Fig. 3). Weed removal was also suggested as part of disease management strategy for the control of tomato spotted wilt virus (TSWV) in southwest Australia where weeds have been shown to act as virus reservoirs (34). Although weeds from nurseries are not known to have been tested, given their presence and the highly susceptible nature of *S. oleraceus* in particular to LBVD, these weeds in nurseries are a suspected source of inoculum (No. 2, Fig. 3). This further supports the management of weeds already included in the disease management strategy for LBVD (29) and also suggested by Navarro et al. (46). While it is also possible that weeds or weed seeds infected with LBVD might sometimes enter the seedling nursery environment in potting mix purchased by the production nursery, thereby introducing the virus (No. 3, Fig. 3), this remains to be determined (Fig. 3). Potting mix and peat purchased by nurseries for composting and use in their commercial mix have been shown to be a source of LBVD inoculum (36) (Fig. 3).

Previous studies done on the occurrence of LBVaV and MLBVV separately on individual weed plants have only included *S. oleraceus* (46) and found that LBVaV occurred as a single infection in six of the plants tested, while MLBVV was never found as a single infection but only as a combined infection with LBVaV in four of the plants tested. *S. oleraceus* and *S. asper* have been confirmed as hosts for LBVD in the United States (4), Australia (39; Fig. 4), and on *S. oleraceus* in Spain (46). It would be valuable to know of any interaction between the two viruses within other weed species, as this would provide further knowledge regarding LBVD epidemiology and may have additional implications on weed removal management strategies. It is also noteworthy that LBVaV seems latent not only in lettuce but also in the experimental test

Fig. 4. An example of a lettuce big-vein disease (LBVD) infection in weed species *Sonchus asper*. The leaf on the left was grown in soil collected from a field where lettuce showed LBVD symptoms. It shows distinct chlorophyll clearing along the veins. The leaf on the right is from a negative control plant grown in sterile potting mix and shows no chlorophyll clearing. Both plants were grown in a controlled environment room at 18°C.

hosts *Nicotiana benthamiana* and *N. clevelandii* (27). It is also not known, as with lettuce, whether the presence of this virus may predispose the weeds to MLBVV infection, which may be important in the epidemiology of LBVD.

Disinfection of soil and potting mix, surface sterilization of seed, and the use of clean water and clean production areas may all be important in the management of LBVD in commercial seedling production nurseries in southwest Australia (36), a region which also experiences a Mediterranean-type climate similar to that of California (60). Latham et al. (36) also showed that contaminated potting mix is a source of inoculum for lettuce seedling nurseries (No. 3, Fig. 3), and even though not practical on a large scale, Maccarone (39) demonstrated that autoclaving potting mix three times at 120°C for 40 min is effective in eradicating *O. virulentus* resting spores. Sterilization of potting mix and soil within commercial seedling nurseries was suggested by Latham et al. (36) and Jones (29) as an important disease management strategy.

Water is also a possible source of LBVD in seedling nurseries, and the reduction of LBVD transmitted through water (No. 4, Fig. 3) could be managed by the use of filtration, which has been used successfully by nurseries to manage the spread of *Phytophthora* spp. (67). Alternatively, water may be decontaminated by chlorination or by exposure to UV light before coming into contact with susceptible plants (61,65). In addition, another source of LBVD inoculum both in the nursery environment and in the field could be wind dispersal if *O. virulentus* is carried in windborne dust (Nos. 5 and 13, Fig. 3) and also with viruliferous resting spores being associated with dry crop debris within the seedling nursery (29) (No. 6, Fig. 3).

Disease Management – Lettuce Growing Fields

As for commercial lettuce seedling nurseries, LBVD can enter commercial lettuce growing fields via a number of routes (Fig. 3). The primary source of inoculum is with the infested field soil commonly used for seedling production (No. 7, Fig. 3), and the level of inoculum in infested field soil may also be increased if there is dry crop debris left in the field (No. 11, Fig. 3). As previously discussed, weeds are a source of inoculum in lettuce growing fields, but given that the seeds of *S. oleraceus* have a similar morphology to lettuce seeds, for example longitudinal ribs along the surface (Fig. 5), it is reasonable to speculate that weed seeds could also be a source of LBVD inoculum in lettuce growing fields (No. 8, Fig. 3). Contaminated weed seeds could transmit LBVD not only to lettuce but also to other susceptible weed species including *A. calendula* and facilitate both the spread and the persistence of LBVD in the field. It is also possible that, as seen with studies done on *Phytophthora* (61), *O. virulentus* resting spores carrying LBVaV

Fig. 5. Schematic drawing of a lettuce seed showing the three layers surrounding the embryo, endosperm, integument, and "ribs" on the pericarp. Adapted from Nijse et al. (48).

and/or MLBVV could be transmitted on farm machinery (also suggested by Jones [29]) and on the footwear of farm workers (No. 10, Fig. 3). Some commercial lettuce growing farms investigated in this study cultivate more than one farm in which lettuce is grown, and machinery such as tractors, trucks, and other vehicles are driven onto and between farms without any implementation of disinfection strategies.

If more annual and perennial native plants are discovered to be hosts of LBVaV and MLBVV, even if they are found to be asymptomatic, then that may add another complexity to the LBVD story in the long-term management of the disease. It is possible that native plants which have natural infections of LBVD act as reservoirs and if they are found to be growing in native vegetation close to lettuce growing fields, motile *O. virulentus* zoospores carrying both LBVaV and MLBVV could be transported through water by a flooding event (No. 12, Fig. 3). As with the consideration for contaminated water sources in the nursery environment, the same sterilization or filtration management options discussed above also apply in relation to irrigation water used in lettuce fields (No. 12, Fig. 3). However, this is not likely to be practical where irrigation water is used in large volumes and when irrigation is necessary in dry periods of the year.

Conclusions

In conclusion, it is possible that the “associations” and “arrangements” the *Olpidium* vector has in tripartite interactions with viruses and plant hosts is complex. Such associations and arrangements could be comparable to those which insect vectors are known to have, as evident in the literature. The greater variety of interactions known to be involved with insect vectors are an outcome of a relatively large number of studies focused on insect vectors of human, animal, and plant viruses. The complexity of the host susceptibility to the virus may be related to the attraction of the *Olpidium* vector to the plant host. Fry and Campbell (20) found that *Olpidium* reproduction was more pronounced on a virus-susceptible variety than on those tolerant to it. This is similar to the observation (18) of the attraction of insect vectors to plants with heavy infection of virus.

The adoption of management strategies for commercial lettuce seedling nurseries and lettuce growing fields as outlined in this review may reduce LBVD incidence and spread with a final objective to increase yield and productivity for lettuce growers, while providing consumers with higher quality produce. Further investigation regarding the nature of these tripartite *Olpidium*–plant–virus interactions may help to develop more targeted management strategies for virus diseases associated with *Olpidium* vectors.

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ERRATUM

On 26 November 2013, this article was modified. The caption for Figure 3, on page 704, was changed to add a citation for Jones. Also, the Acknowledgments, on page 706, were changed to add additional information and acknowledge additional supervisors.