Protected tomato – A review of root mat and crown gall diseases to inform research on control of tomato root mat

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Contents

Background

Root mat and crown gall are both members of a small group of unusual plant diseases (neoplastic diseases) in which the infecting bacterium, usually a species of *Rhizobium*, causes new plant tissues to grow. The induction of root mat or crown gall symptoms is a unique and highly specialised process involving gene transfer from the infecting bacterium to the plant (i.e. trans-kingdom). Both root mat and crown gall occur naturally in tomato.

Tomato root mat disease was first observed in the UK in 1999 and the following year symptoms were shown to be caused by a rhizogenic (i.e. root-inducing) strain of the bacterium *Rhizobium radiobacter* (previously *Agrobacterium* bv. 1) (Weller *et al*., 2000). The disease causes massive over-production of roots which results in increased vegetative growth, reduced fruit yield and difficulty with crop management. It has affected crops grown in various growing media and both own root and grafted plants. Once present on a nursery the disease tends to persist for many years. Although crop management practices can partially alleviate effects on yield and increase plant survival, there are no proven treatments available to prevent infection or reduce symptom expression. Tomato root mat appears to be increasing in importance both in the UK and overseas.

Tomato crown gall was first reported in commercial crops in the UK in 2006 (Weller & O'Neill, 2006). It remains a relatively rare disease on tomato and has not caused concern. Crown gall is however a significant disease problem of various Rosaceous crops and herbaceous perennials, both in the UK and worldwide.

Crown gall diseases, caused by tumorigenic strains of *Rhizobium* species (previously *Agrobacterium*), have been studied considerably more than root mat at both the crop/disease level and the cell/molecular level. The literature on crown gall and its control may therefore provide insights on tomato root mat epidemiology and help to identify the most promising potential control options.

The aims of this technical review were: 1) to summarise current knowledge on tomato root mat and 2) to identify potential research areas to improve understanding and management of the disease. The expected deliverables were: 1) a sound body of information as a basis to produce the text for a factsheet on tomato root mat; 2) a review of previous research on root mat to inform the proposed research programme; 3) identification of knowledge gaps pertinent to the control of tomato root mat.

Summary

Losses due to root mat disease on one 26ha UK nursery in 2009 were estimated at around 0.75 million pounds per year. Losses arise due to increased costs of crop management, an increased proportion of fruit being out of specification, and an increased susceptibility of transformed plants to secondary root diseases. Root mat in tomato is caused by rhizogenic plasmids (pRi), and crown gall is caused by tumorigenic plasmids (pTi), most commonly vectored by *Rhizobium radiobacter*, a common soilborne bacterium. Bacteria causing root mat and crown gall may both acquire and lose these plasmids. Bacteria may also carry a varying number of additional plasmids. There is no evidence that Ri and Ti plasmids in the absence of bacteria will cause disease symptoms. Recently, the genus *Rhizobium* was revised to incorporate all species previously described as *Agrobacterium*. This classification was based on 16S ribosomal DNA analysis and hence genetic relatedness. The genus *Agrobacterium*, originally established to contain plant pathogenic species closely related to *Rhizobium*, was considered to be an artificial genus.

In this review we use the current accepted name *Rhizobium radiobacter*, with the qualifying descriptor 'rhizogenic strain', to identify the cause of tomato and cucumber root mat. Where we report on experimental work on different crop species, and crown gall rather than root mat, we have generally retained the original *Agrobacterium* species name as given in the particular reference in order to avoid introducing possible errors (there is not a unique one-to-one translation between *Agrobacterium* and *Rhizobium* species names) and unnecessary complexity.

The development of crown gall (and also likely root mat) is activated by fresh wounds on roots or stems which produce exudates that act as signal molecules; bacteria move to the wound site along the chemical gradient. Infection occurs when a piece of the plasmid DNA, known as the transferred DNA (T-DNA), is transferred from the bacterium and incorporated into the host plant nuclear DNA. Genes contained on the plasmid are expressed when inserted into the plant genome leading to a plant hormone imbalance that results in tumour growth (crown gall) or uncontrolled root proliferation (root mat) at the infection site. Infected plant cells synthesise simple novel metabolites, known as opines, that are not found in normal plant tissues. The pattern of opines synthesised is determined by the type of virulence plasmid in the bacterium and, in general, the virulence plasmids also confer on the infecting bacterium the ability to utilise the same opines as nutrients.

In inoculation experiments, both inoculum concentration and plant age have been found to influence infection success and severity of symptoms. Tomato root mat disease does not appear to spread rapidly between plants. It is quite common to find rockwool or coir slabs

with one plant severely affected and other plants in the same slab displaying no symptoms. Possibly this is because plants become less susceptible to transformation by the Ri plasmid as they age. Substrate type has also been found to affect root mat and both incidence, and severity of symptoms has been observed to differ between different types of coir. There is relatively little information on the effect of imposed tissue wounding on susceptibility of plants to root mat. The literature on crown gall clearly demonstrates that tissue wounding is important for infection of plants by tumorigenic *R. radiobacter*, and although experiments to date have not demonstrated a requirement for imposed root damage to permit development of root mat in tomato or cucumber, it is possible that the experimental procedures used for growing the plants have produced sufficient root damage to allow infection, or natural wound sites around emerging lateral roots provide the infection court.

Once a plant is infected with the Ri or Ti plasmid, there are no known treatments which will prevent symptom development. Consequently, the current focus for control of both root mat and crown gall disease is to prevent infection. As with other plant diseases, this may be achieved by host resistance, by environment manipulation to make conditions unfavourable for infection, or by reduction/elimination of rhizogenic *R. radiobacter* inoculum in the environment around plants. Most of the tomato varieties and rootstocks currently grown in the UK appear to be susceptible to root mat. Cultivar resistance to crown gall has been reported (e.g. in rose) and one tomato variety (cv. Kanavaro) has been observed to be less susceptible to root mat than others. It is possible that increased cultivar resistance to root mat in tomato may be identified. No root zone environment manipulation treatments that reliably reduce root mat have yet been identified. There is speculation that oxygen level in irrigation solution and irrigation frequency may influence the disease. There is good reason to believe biological treatments could reduce tomato root mat by influencing the population of rhizogenic bacteria around tomato roots. Specifically, recent work on crown gall disease showed that a quorum sensing signal is produced by populations of *A. tumefaciens* that controls transfer of the Ti plasmid. Transfer of the Ti plasmid only occurs at high population densities of *A. tumefaciens*, when concentration of the signalling molecule is high. Various isolates of *Bacillus*, *Pseudomonas* and *Trichoderma* species have been shown to reduce crown gall, possibly through reduction of *A. tumefaciens* populations. Assuming quorum sensing also operates with root mat disease, biological products might reduce root mat if they prevent the population reaching a threshold concentration where plasmid transfer occurs. Modified strains of *Agrobacterium* have shown most promise in control of crown gall and some (e.g. Galltrol) are marketed for this purpose, although not in the UK; *Agrobacterium radiobacter* K84, the active ingredient of Galltrol is considered to be a genetically modified organism by regulatory authorities and currently this prevents registration in the UK. Previous trials with biological

products for control of root mat were largely unsuccessful due to low incidence and/or high variation in disease occurrence. A number of products, the majority of which are biological, were tested in primary screens at ADAS Boxworth in 2016.

Various knowledge gaps pertinent to the control of root mat were identified and are listed below as a series of questions.

Sources of infection

1. Does rhizogenic *R. radiobacter* occur on commercial batches of tomato seed?
2. Is rhizogenic *R. radiobacter* present in irrigation water or growing media on propagation nurseries? Or associated with sciarid flies or other insects that frequent the tomato root zone?
3. Can the Ri plasmid persist in the environment in the absence of *R. radiobacter* or other vectoring bacteria?
4. Is there latent root mat infection in tomato plants at receipt on production nurseries?

Control by host resistance

5. What is the relative susceptibility to infection of:
    - Seedlings germinating in plugs (propagation nursery)
    - Young plants growing in cubes (propagation nursery)
    - Young plants rooting into slabs (production nursery)
    - Plants well established on slabs (production nursery)?
6. Is there a useful level of resistance to root mat in any tomato genotypes?
7. Can induction of host resistance (Systemic Acquired Resistance or Induced Systemic Resistance) in tomato provide any control of root mat?

Control by inoculum reduction

8. How effective are microorganisms, biological preparations and biocides at maintaining rhizogenic *R. radiobacter* at nil or low population levels in the root zone and the wider glasshouse environment?
9. Does hypochlorite treatment of tomato seed for *Pepino mosaic virus* adequately control any *R. radiobacter* on/in seed?

Control by environment manipulation

10. Can we reduce opine accumulation to deprive *R. radiobacter* of nutrition and prevent population increase?
11. Does handling of plug plants or propagation blocks result in root damage sufficient to significantly influence susceptibility to infection? If so, can handling practices be adapted to minimise root damage and reduce infection?
12. Can we mask/interfere with phenolic compounds produced by tissue wounds and thereby reduce movement of rhizogenic *R. radiobacter* towards susceptible root tissue?
13. Does hypochlorite treatment of tomato seed increase susceptibility to infection by rhizogenic *R. radiobacter* by removal of non-pathogen strains and/or other competing microorganisms?
14. Would application of non-pathogenic microorganisms to seeds soon after hypochlorite seed treatment, especially root colonising bacteria, reduce the susceptibility of young plants to root mat, for example by colonisation of natural wound sites where lateral roots emerge?
15. Does irrigation solution temperature, pH, oxygen level, conductivity, nutrient form or level significantly influence the susceptibility of tomato roots to infection by rhizogenic *R. radiobacter*?
16. Does the water holding capacity of a slab, profile of water distribution in a slab, or irrigation frequency, influence susceptibility of tomato plants to root mat?
17. Do environmental and crop management actions directed at switching plants from generative to vegetative growth increase susceptibility to root mat? Does induction of vegetative growth result in increased lateral root production?

Technical review

1. Occurrence of root mat

Root mat disease occurs naturally in relatively few plant species: apple, aubergine, cucumber, melon, rose spirea, and tomato (Veena & Taylor, 2007; De Cleene & De Ley, 1981). In apple and rose the disease caused by *R. radiobacter* is generally termed hairy root whereas in cucumber it is generally termed root mat. In tomato it is known as root mat in the UK and, more recently, in continental Europe and Canada it has been called ‘crazy roots’.

a) Rosaceous crops

A problem known as hairy root syndrome or root mat disease caused by a bacterium was described in apple more than 80 years ago (Riker *et al.,* 1930; Hildebrand, 1934). The causal bacterium was identified as *Phytomonas rhizogenes*, later re-named *Agrobacterium rhizogenes*, and more recently *Rhizobium radiobacter* (see section 3). The bacterium is a close relative of *Agrobacterium tumefaciens*, the causative agent of crown gall disease and the best-characterised species among the genus *Agrobacterium*.

Hairy root was first shown to be an infectious disease in apple in the USA (Riker *et al.,* 1930). The disease developed naturally around the graft wound at soil level, appearing as a large number of lateral fleshy roots, initially unbranched but later branched and sometimes fibrous (Riker & Hildebrand, 1934). Around the same time spirea was reported as a natural host (Suit, 1933). In field roses, a disease known variously as hairy root, bristle root and hairy gall because of variable symptomatology, was described in the USA in 1937 (Hildebrand, 1937) and reached epidemic proportions in California in the 1950s (Munnecke *et al*., 1963). Profuse root growth occurred on the base of cuttings, on stem wounds and roots, and in the second year growth of affected field roses was poor and many plants died. *Agrobacterium rhizogenes* was shown to be the cause of hairy root in rose, causing tremendous stimulation of new root growth from the cambium.

Although the natural host range of rhizogenic *R. radiobacter* (*A. rhizogenes*) appears to be restricted to a small number of plant species, under laboratory conditions 37 of 202 inoculated species were found to be susceptible (De Cleene & De Ley, 1981). Thirty three of these 37 species belonged to the closely related subclasses Asteridae and Rosidae (Table 1). It was noted that there are indications that families which are typical polyphenol accumulators are more susceptible to the disease. None of 16 monocots, three ferns and one gymnosperm were found to be susceptible. None of the inoculations with A*. rhizogenes* resulted in secondary hairy root formation away from the local wound site (De Cleene & De Ley, 2007).

Table 1. Plant species reported to develop root mat symptoms following inoculation with a rhizogenic strain of *Agrobacterium rhizogenes* (adapted from De Cleene & De Ley, 1981).

Susceptible following wound inoculation (192 tested)		**Susceptible on intact hosts (13 species tested)**	
Family	**Species**	**Family**	**Species**
Juglanaceae	*Juglans* sp.	Chenopodiaceae	*Beta vulgaris*
Moraceae	*Morus abla*	Crassulaceae	*Kalanchoe* spp.
Chenopodiaceae	*Beta vulgaris*		*Sedum* sp.
Ranunculaceae	*Delphinium* sp.		*Malus sylvestris*
Crassulaceae	*Kalanchoe* spp. (x3)	Solanaceae	*Lycopersicon* sp.
	Sedum spp. (x2)		
Rosaceae	*Cotoneaster acuminatus*		
	Cydonia oblonga		
	Geum reptans		
	Malus sylvestris		
	Prunus persica		
	Pyrus pulcherrima		
	Rosa spp. (x2)		
	Rubus idaeus		
	Spiraea spp. (x2)		
Leguminosae	*Caragana arborescens*		
	Gleditsia triacanthos		
	Laburnum alpinum		
	Phaseolus vulgaris		
	Vica faba		
Geraniaceae	*Pelargonium zonale*		
Balsaminaceae	*Impatiens balsamina*		

Eleagnaceae	*Eleagnus angustifolia*		
Umbelliferae	*Daucus carota*		
Labiateae	*Coleus blumei*		
Solanaceae	*Lycopersicon lycopersicum*		
	Nicotiana tabacum		
Caprifoliaceae	*Lonicera* spp. (x3)		
	Symphoricarpus racemosus		
Compositae	*Chrysanthemum frutescens*		

Notes: 1) No cucurbit species were tested in this work; 2) Pepper (*Capsicum annuum*) was inoculated and no root mat symptoms developed; 3) Aubergine was not tested.

b) Cucumber

Cucumber root mat disease was first described in the late 1970s when it appeared as a mystery problem affecting many soil and straw-bed grown cucumbers in the Lee Valley (Yarham & Perkins, 1978). Typically, roots grew upwards on the soil or bed surface in a dense mat (Figure 1). When plants were removed, thickened, knotted roots could be found for considerable distances through the bed, unbranched at first, but eventually ending in a mass of very fine roots. Experimental work at the time indicated the disease was caused by rhizogenic *R. radiobacter* (D J Yarham, pers comm.). Within a few years the disease disappeared as mysteriously as it had arrived.

The disease re-appeared in 1993, affecting crops grown on rockwool slabs, initially in a crop of cv. Jessica in Berkshire (O'Neill & Yarham, 1993). Rhizogenic *R. radiobacter* was confirmed as the causal agent by completion of Koch's postulates (O'Neill, 1994). In 1993 symptoms on cucumber occurred in mid-February and around 2 months after planting. Fine roots were found growing upwards from the rockwool propagation cube. By May, some cubes had masses of small erect roots, up to 10 mm tall, growing upwards from the cube surface; sometimes roots were matted together around the drip nozzle (Figure 2). Removal of the plastic slab wrapper revealed a dense mat of thin, bright white, mostly unbranched roots (Figure 3). This proliferation of roots swelled the cube and sometimes the slab out of its normal shape. In some slabs, very thick corky roots, up to 10 mm diameter, developed (Figure 4). In both the soil and straw bed crops in the 1970s, and in rockwool crops from

1993, development of root mat symptoms on plants was associated with increased vegetative growth and reduced fruit yield and quality – young fruit damped off, there was an increase in the proportion of male to female flowers and an increased number of bent fruit. Root mat affected many rockwool cucumber crops in England over the following two decades. Gradually, however, the disease has become less common in cucumber, associated with and probably due to the move to replanting crops twice each year; there is generally insufficient time for significant symptoms to develop in the resultant short term (8-12 week) crops.

Cucumber root mat has also been reported in France (Weller *et al*., 2006), Russia (Ignatov *et al*., 2015) and The Netherlands (van Marrewijk & Vermunt, 2010).

Figure 1. Root mat symptoms in cucumber, 1978

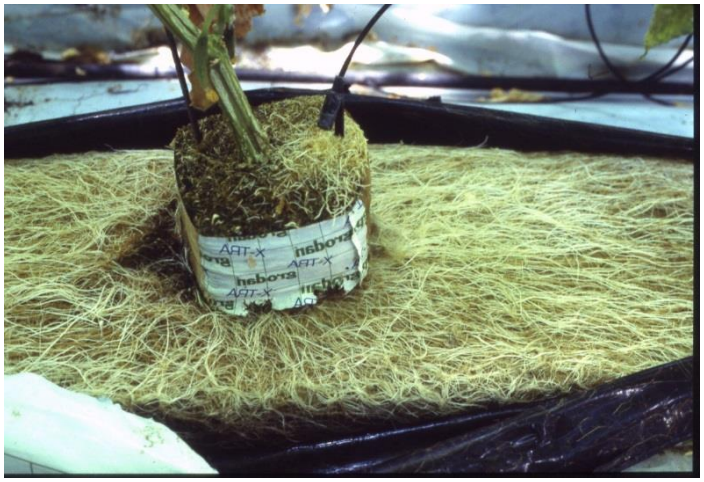

Figure 2. Excess root growth over the slab surface and upwards around the drip nozzle in cucumber, 1993

Figure 3. With cucumber root mat most of the proliferating roots are unbranched

Figure 4. Thickened corky roots sometimes develop with both cucumber root mat (above) and tomato root mat

c) Tomato

Root mat disease in tomato was first observed in 1999, in the UK, on a batch of plants propagated in the Netherlands. The disease was confirmed in 2000 when symptoms were shown to be caused by *Rhizobium radiobacter* (previously *Agrobacterium* bv. 1) harbouring a root-inducing (pRi) plasmid (Weller *et al*., 2000).

The predominant symptom in tomato is extensive root proliferation within the propagation cube and across and within the slab. Roots grow upwards out of the top of the propagation cube, commonly around the irrigation peg, and within the cube and slab causing swelling and distortion. Drainage channels may become blocked by the excessive root growth. Thickened roots occasionally occur (O'Neill, unpublished; Sawada & Azegami, 2014), as with cucumber (Figure 4).

Several detrimental effects of root mat disease on crop growth and fruit production in tomato have been observed. Irrigation becomes difficult as water runs off the top of cubes with dense root mat; root rots caused by *Pythium* spp. and *Fusarium oxysporum* often occur within the slab and plants may lose vigour, wilt and die (T O'Neill, unpublished). Investigations on a cherry tomato crop indicated that fruit diameter of king fruit was reduced by 2-3 mm leading to a higher proportion of unmarketable fruit (O'Neill, 2009; Weller *et al*., 2000). There was no obvious effect on fruit number per truss but yield was reduced by 15%. Growers report that plants become more vegetative and the fruit may be bladdery and of poor taste. Stems are reported to become thicker and leaves larger, there may be abortion of flowers, reduced fruit set and kinked trusses (Van Kerckhove, 2015). The incidence of affected plants in a crop can vary greatly, from <1% to 50%. Recent information from Belgium indicates that yield losses can range from 5-15% (Andy Lee, Grodan, pers. comm). As a tomato crop is worth £500K to £750K per hectare this represents a very large cost, especially as profitability is very low as a % of turnover for most growers. The disease has been recorded on a wide range of varieties and rootstocks; and in crops grown on rockwool, coir, NFT and in soil. In 2009, it was estimated that losses due to root mat on one 26ha UK tomato nursery were in the region of £0.75 million/annum, due to increases in secondary diseases and crop management costs (McPherson, 2009). Consequently any reduction in the level of root mat would offer a significant benefit.

In Belgium, occurrence of root mat in tomato has increased from 8% of crops pre-2011 to 26% of crops in 2012 (Van Kerckhove, 2015).

In Belgium, occurrence of root mat in tomato has increased from 8% of crops pre-2011 to 26% of crops in 2012 (Van Kerckhove, 2015).

Root mat disease of tomato (and cucumber) has recently been reported in Russia (Ignatov *et al*., 2015), where it was first found in winter 2013-14 in a newly constructed glasshouse on crops grown in rockwool with disease incidence ranging from 50-100% of plants. Root mat of tomato was first observed in Japan in 2011 (Sawada & Azegami, 2014), in a crop grown on coir slabs with recirculation of irrigation solution. The disease had previously been reported in Japan on melon (see section 1d).

The emergence of root mat as a serious disease of hydroponic cucumber and tomato crops in numerous countries around the world is currently unexplained. *R. radiobacter* appears to be systemic in both cucumber and tomato plants (see later) and rhizogenic strains of the bacterium were isolated from tomato fruit (Ignatov *et al*., 2015). There are reports of seedborne infection by *A. tumefaciens* in *Prunus*, *Humulus* and *Brassica* (Richardson, 1990; Weller et al., 2002). There is circumstantial evidence that the disease may arise in

propagation, as for example when plants received from only one of several propagators on a production nursery are affected. Experimental work indicates that younger plants are generally more susceptible to infection than older plants suggesting that propagation is a high risk period. Possibly changes in production practices during propagation (e.g. introduction of ebb-flood irrigation) have produced a root environment more conducive to infection. International trade of young plants may also have contributed to the emergence of root mat as an important disease in countries close to each other (e.g. within Europe), though unlikely between Europe, Russia, Japan and Canada where there is generally no trade in tomato plants. Further work is required to elucidate the relative importance of seed transmission, infection during propagation and movement of young plants, and possibly other factors, in the emergence of tomato root mat as a serious disease problem in Europe, Asia and North America over a relatively short period.

d) Other protected edible crops

In Japan a new disease of greenhouse melons, characterised by symptoms of hairy roots, was observed from 1976. Bacterial isolates obtained from hairy roots were pathogenic to melon by needle prick inoculation and developed symptoms similar to those caused by natural infection; the bacterium was identified as *Agrobacterium rhizogenes* biovar 1 (Shiomi *et al*., 1987).

In the Netherlands root mat is reported to be a serious problem in aubergine (*Solanum melanogea*), occurring on both own root and grafted plants. Rootstocks affected include Emperador, Efialto and Maxifort. We are not aware of any reports of root mat on pepper. Pepper was examined as a host by De Cleene and De Ley (1981) and no symptoms had developed 226 days after wound inoculation of the stem, and so would appear to be non-susceptible.

2. Occurrence of crown gall

A bacterium termed *Bacillus ampelosporae* was first identified as the cause of crown gall disease, in grape, in 1897. This organism, subsequently termed *Agrobacterium vitis*, causes the growth of neoplastic tumours on the stem and crown of grapevine. *A. vitis* can survive in intercellular spaces in plants without causing disease, but will initiate tumorigenesis on tissue wounding (most commonly frost injury) (Burr *et al*., 1998). Around the same time, *Bacterium tumefaciens* (now *Agrobacterium tumefaciens*) was reported as the causal agent of crown gall disease in Paris daisy (Smith & Townsend, 1907). This organism is capable of inducing tumours at wound sites on the stems, crowns and roots of hundreds of dicot species from many different plant families.

Although crown gall disease is not generally fatal unless infection occurs in young plants, reductions in crop yield and/or vigour can be significant in many perennial horticultural crops, including grape, apple and cherry (Escobar & Dandekar, 2003). In addition, crown galls are sites for secondary infections by other plant pathogens or pests and can increase plant susceptibility to abiotic stresses.

a) Rosaceous crop

Crown gall disease is most frequently found on members of the rose family although it can affect species in over 90 different plant families (Table 2) (De Cleene & De Ley, 1976), a marked contrast to the limited natural host range reported for root mat disease. The molecular and genetic basis for the host range of different *Agrobacterium* (*Rhizobium*) strains is unclear (Gelvin, 2003). It is a complex process under the genetic control of multiple factors within both the bacterium and the plant host (Gelvin, 2003).

Table 2. Plant families reported as natural hosts of crown gall disease (adapted from De Cleene & De Ley, 1976)

Abies	*Dianthus*	*Laburnum*	*Populus*	*Spiraea*
Achillea	*Elaeagnus*	*Lantana*	*Primula*	*Solidago*
Actinidia	*Erica*	*Ligustrum*	*Prunus*	*Solanum*
Allium	*Eucalyptus*	*Lonicera*	*Pyracantha*	*Sorbus*
Alnus	*Euonymus*	*Malus*	*Pyrus*	*Stachys*
Ampelopsis	*Euphorbia*	*Morus*	*Ranunculus*	*Symphoricarpus*
Anemone	*Ficus*	*Malva*	*Ribes*	*Syringa*
Anthemis	*Forsythia*	*Nepeta*	*Rhododendron*	*Tagetes*
Arabis	*Fraxinus*	*Oenothera*	*Rheum*	*Taxus*
Aster	*Fuchsia*	*Oxalis*	*Rosemary*	*Thuja*
Begonia	*Genista*	*Pachysandra*	*Rubus*	*Thunbergia*
Camellia	*Gleditsia*	*Parthenocissus*	*Rosa*	*Tilia*
Castanea	*Gypsophila*	*Penstemon*	*Rudbeckia*	*Ulmus*
Cherianthus	*Hedera*	*Pinus*	*Rumex*	*Vaccinium*
Clematis	*Hibiscus*	*Philadelphus*	*Ruta*	*Verbascum*
Cordyline	*Humulus*	*Philodendron*	*Salix*	*Verbena*
Crataegus	*Hypericum*	*Phlox*	*Salvia*	*Veronica*
Dahlia	*Ilex*	*Physalis*	*Scabiosa*	*Vinca*
Daphne	*Jasminum*	*Picea*	*Sedum*	*Wistera*
Datura	*Juglans*	*Platanus*	*Silene*	*Zinnia*

In the UK, the rosaceous crops most commonly affected are apple, blackberry, cherry rootstock (especially Colt), other *Prunus* species, pear and rose.

b) Other plant families

The disease is quite common in chrysanthemum. In a survey of UK HNS and herbaceous perennial growers in 2009, crown gall was reported as occurring in 14 of 16 crops listed: anemone, aster, camellia, cherianthus, chrysanthemum, euonymus, fraxinus, gleditsia, gypsophila, juglans, lonicera, salix and vaccinium; only two of the 16 listed crops were reported by growers not to be affected - clematis and dahlia (Adlam & O'Neill, 2009).

3. Classification and nomenclature of *Agrobacterium* and *Rhizobium*

The taxonomy of the bacteria causing root mat and crown gall is complex and can be confusing. This is because names of the causal bacteria have changed several times, as new information has become available and the now redundant names *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens* are still commonly used by some authors. Also, the specific names *rhizogenes* (root inducing) and *tumefaciens* (tumour forming) have been retained for bacteria which may not produce root mat and crown gall symptoms in plants; root mat and crown gall symptoms are induced by plasmids transferred from bacteria to the host, and not by the bacteria themselves. Root mat is caused by rhizogenic plasmids (pRi), and crown gall is caused by tumorigenic plasmids (pTi). Some bacterial species are capable of carrying both pRi and pTi (we found no reports of pRi and pTi being carried simultaneously). Furthermore, the bacteria causing root mat and crown gall may both acquire and lose these plasmids. Culture of bacteria at 39°C can lead to plasmid loss. There is no evidence that Ri and Ti plasmids in the absence of bacteria will cause disease symptoms.

For many years the accepted name of the bacterium causing root mat was *Agrobacterium rhizogenes* and that of the bacterium causing crown gall was *Agrobacterium tumefaciens* (Hayward & Waterston, 1965a; Hayward & Waterston, 1965b). Earlier synonyms for *A. rhizogenes* were *Phytomonas rhizogenes, Bacterium rhizogenes* and *Pseudomonas rhizogenes* (Hayward & Waterston, 1965a); these are now redundant. However, as it was realised that *A. rhizogenes* can be 'converted' into *A. tumefasciens* simply by substituting one type of plasmid for another, these species names became meaningless (Gelvin, 2003).

Subsequently, *Agrobacterium* species were re-classified as three biovars, 1, 2 and 3 (Keane *et al*., 1970; Kerr & Panagopouloss, 1977, Young *et al*., 2001) (Table 3). This classification was based on numerical analysis of phenotypic characteristics including biochemical and physiological tests, fatty acid profiles, electrophoresis of soluble proteins and thermal stability of DNA–DNA hybrids. The results obtained by all methods indicated three distinct groups,

not including *Agrobacterium ribi*, these are known as biovars (or biotypes) 1, 2 and 3. Under this grouping, all *A. rhizogenes* strains are classified as either biovar 1 or biovar 2.

More recently, the genus *Rhizobium* was revised to incorporate all species previously described as *Agrobacterium* (Young *et al*., 2001; Young *et al.,* 2003). This classification was based on 16S ribosomal DNA analysis. The DNA analysis showed that all *Agrobacterium* species should be amalgamated into the *Rhizobium* genus; *Agrobacterium* was considered to be an artificial genus comprising plant pathogenic species. Although this proposal was challenged by Ferrand *et al* (2003), the challenge was defended (Young *et al.*, 2003). The re-classification of *Agrobacterium* species as *Rhizobium* species has now been accepted in the Comprehensive list of names of plant pathogenic bacteria, 1980-2007 (Bull *et al*., 2010).

The effect of this re-classification on the names of bacteria associated with root mat and crown gall diseases, previously referred to as *Agrobacterium*, is summarised in Table 4. The correct name of the principal bacterium causing root mat in cucumber and tomato is now *Rhizobium radiobacter*; that of the bacterium causing root mat/hair root in apple and rose is *Rhizobium rhizogenes*. It should be noted that *R. radiobacter* may also cause crown gall or no symptoms.

Table 3. Biovar classification of *Agrobacterium* species*

Biovar	**Type species of this biovar**	**Features**
1	*A. tumefaciens* strain C58	1 large circular chromosome 1 large linear chromosome Ti plasmids Helper plasmids
2	*A. radiobacter* strain K84	1 large chromosome 1 megaplasmid (2.65 Mb) Several smaller plasmids
3	*A. vitis* strain S4	2 large circular chromosomes 1-5 smaller plasmids
1 or 2	*A. rhizogenes* strain A4	1 large chromosome Several smaller plasmids

* Information taken from Veena & Taylor, 2007.

Table 4. *Rhizobium* genus as revised by Young *et al*., (2003) showing those species causing crown gall or root mat/hairy root.

***Rhizobium* species**	**Plasmids reported in this species**	**Symptoms**	**Hosts**
R. radiobacter	Nil	-	-
	Ti	Crown gall	Many
	Ri	Root mat	Cucumber, tomato, melon
R. rhizogenes	Nil	-	-
	Ti	Crown gall	Many
	Ri	Hairy root	Apple & rose
R. rubi	Nil	-	-
	Ti	Cane gall	*Rubus* spp.
*R. vitis**	Nil	-	-
	Ti	Crown gall	Grapevine

*The NCPPB contains some biovar 3 strains obtained from grapevine in Afghanistan which carry the Ri plasmid; however, root mat has not been reported in grapevine.

4. Detection and identification

a) Rhizobium radiobacter

Description

Rhizobium radiobacter is a common soilborne bacterium. The original isolate obtained from infected apple trees was named *Phytomas rhizogenes,* subsequently *Agrobacterium rhizogenes*. It was described as a short, non-sporulating, Gram-negative rod, 0.55 – 0.59 µ (average 1.44 µ) in length and 0.15 – 0.75 µ (average 0.43 µ) in diameter, with a polar flagellum. Later studies showed that *R. radiobacter* has 1-4 peritrichous flagella. The optimum temperature for growth was 20-28°C; no growth occurred below pH 4 (Riker *et al*., 1930).

Recent work on genotyping of *R. radiobacter* based on partial DNA sequencing, suggests that isolates from tomato and cucumber differ phylogenetically; most 'tomato isolates' belonged to genomovar 9 and most 'cucumber isolates' to genomovar 7 (Van Kerckhove, 2015). The same group reported that tomato and cucumber isolates had a temperature optimum of 28ºC (minimum 4ºC, maximum 44ºC), and a pH optimum to 6.5 (minimum pH 4, maximum pH 11). As noted earlier, the presence of Ri or Ti plasmids determines the pathogenic status of strains (rhizogenic, tumorigenic or non-pathogenic (saprophytic)).

Isolation

Non-pathogenic strains of *R. radiobacter* are ubiquitous in soils, and appear to be common in hydroponic solutions used in cucumber and tomato production and associated plant roots and stem bases. A partially selective bacterial growth medium (Scroth's medium) is available to isolate, identify and quantify *R. radiobacter* (*Agrobacterium* biovar. 1) but this does not distinguish pathogenic isolates with root inducing plasmids (Scroth *et al*., 1965). Selective growth media are also available for *Agrobacterium* biovar. 2 (Brisbane & Kerr, 1983) and *Agrobacterium* biovar. 3 (Lelliott & Stead, 1987), referred to as Brisbane and Kerr Medium 2E and Sasser Medium 3 respectively. All the strains obtained from tomato and cucumber examined to date have been biovar 1.

In grapevine, higher populations of biovar 3 of *A. tumefaciens* and *A. radiobacter*, both causal agents of crown gall in grapevine, were isolated from roots of symptomatic vines than from asymptomatic vines. When specific sections of roots were assayed for biovar 3, it was predominantly isolated from small, dark, sunken lesions (Burr & Bishop, 1987). No lesion symptom has been described for early stage root mat in tomato or cucumber.

Heterogeneity of Agrobacterium strains

There is some indication that biovar type affects the host range of rhizogenic strains, although it may not be a strict relationship. All reports to date of natural infection in cucumber and tomato are by *R. radiobacter* biovar 1. Isolates from apple and rose in the NCPPB are biovar 2. The host range of a particular isolate of a particular biotype probably depends on a combination of factors including a) ability to multiply in the rhizosphere and attach to roots, b) compatibility of T-DNA for insertion into the host genome, c) presence/absence of resistance mechanisms in the host genotype.

Subsequent work has indicated that there is heterogeneity within *Agrobacterium* bv 1; some, but not all isolates obtained from UK tomato crops caused symptoms in cucumber, and vice-versa (O'Neill, 2001). Van Kerckhove (2015) stated that the virulence of isolates is related to the isolate itself and is not a result of environmental conditions.

b) Plasmids

The number of plasmids in *R. radiobacter* can vary. Petit *et al*. (1983) reported that 5 of 6 strains of *A. rhizogenes* they examined contained three plasmids, of which the largest was a cointegrate of the two others. Both Ti and Ri plasmids have been classified by size and by the type of opine synthesis they induce (e.g. octopine, nopaline and agropine-type plasmids (Engler *et al*., 1981). Ti plasmids are of the order 200-800 kbp in size (Gelvin, 2003); the Ri plasmids associated with cucumber and tomato root mat in the UK were found to be around

217 kbp in size (Weller *et al.,* 2006). Sequencing work in PE 029 will further examine variation between Ri plasmids. Sciaky *et al*. (1978) and Drummond (1979) reported that only one of multiple plasmids in *R. radiobacter*, the Ti plasmid, is essential for crown gall formation. Pulawska *et al*. (1998) studied diversity of plasmids isolated from *A. tumefaciens* originating from fruit trees. Plasmids varied in size from 27-315 kbp and numbered from one to four per isolate. There was no correlation of plasmid number or size with virulence, biovar designation, host or geographical origin. Sawada & Azegami (2014) reported that the cucumopine Ri plasmids harboured by *R. radiobacter* isolates in Japan and Europe were highly homogenous, suggesting the plasmids derived from the same origin.

A qPCR test developed at Fera is available (Weller & Stead, 2002) to determine if *R. radiobacter* isolates contain Ri plasmids. However, not all variants of this plasmid are detected using the assay. It is therefore unclear whether the assay can be reliably used to detect plasmid DNA incorporated into transformed roots of tomato and cucumber plants, where the rhizogenic bacteria may no longer be present, before symptoms of root mat have developed.

The availability of a reliable qPCR test able to detect the known diversity of Ri plasmids would both permit accurate evaluation of infection (including pre-symptomatic) and strengthen reliability of results from work investigating efficacy of control measures. Further sequence analysis of plasmids from different isolates and full test validation for detection of transformed tomato root tissues is required. Such a test would allow better determination of when infection occurs during plant growth. Depending on the discovery of conserved and diagnostically useful T-DNA sequences, tests may involve a laboratory based PCR assay and/or a LAMP assay with added potential for use on nurseries by growers. It would also permit an investigation of the efficacy of control measures and other factors affecting symptom expression by infected plants. It is possible that plants carrying the Ri plasmid do not automatically develop root mat symptoms and certain environmental or other triggers may be needed to switch-on the genes. Genomic sequence comparisons of different bacterial populations may also be useful in the examination of sources and pathways of infection.

5. Sources of infection

Non-pathogenic strains of *R. radiobacter* (i.e. those without the Ri plasmid) are commonly found in hydroponic tomato crops occurring in the solution around roots, in soil and in pooled water. In previous work on commercial cucumber and tomato nurseries (O'Neill, 2001) we detected *R. radiobacter* biovar 1 on concrete pathways, trolley wheels, knives and sciarid flies; in roots and stem base tissue of tomato plants and occasionally from internal stem tissue near the plant head (Table 5).

Table 5. Detection of *R. radiobacter* at different locations in cucumber glasshouses where the crop was affected by root mat – 1998

Swab samples positive for *R. radiobacter*	**Number positive/ number tested**	**Swab samples negative for *R. radiobacter***	**No. samples tested**
Soil - outside	10/20	Glass	1
- inside	9/25	Aluminium support	5
Root pieces	4/4	Foot dip (disinfectant)	1
Run-off solution	9/16	Applied irrigation water	1
Rockwool block	9/20	Cucumber seed	1
Rockwool slab	2/10	Steamed RW slab	4
Irrigation pegs	6/20	New cane	1
Concrete floor	3/8		
Moss/algae	1/1		
Trolley wheel	1/2		
Sciarid fly	4/5		
Sciarid larvae	2/2		
Used canes	1/1		
Organic feed	1/1		

Providing the Ri plasmid is not present on a nursery, the presence of *R. radiobacter* will not result in root mat disease. Initial outbreaks most probably arise from a batch of infected or contaminated plants or trays brought onto a nursery, or from locally infested soil, or soil water, gaining entry to the root zone. On nurseries where growers buy plants from several propagators, in some years observations strongly indicate an association with a specific propagator.

Non-pathogenic *Agrobacteria* are frequently found as endophytic bacteria (Cubero *et al*., 2006, Yakobe *et al*., 2012) and have been discovered in *Brassica napus* seeds (Weller *et al*., 2002).

Recently, rhizogenic *R. radiobacter* biovar 1 was isolated from tomato seed of fruit collected from root mat of infected plants (Ignatov *et al*., 2015).

6. Infection process and symptom development

A diagramatic lifecycle of infection by rhizogenic *R. radiobacter* to cause root mat symptoms is proposed below (Figure. 5). Our understanding of the molecular mechanisms of genetic transformation of plants by *Rhizobium* (*Agrobacterium*) species relies on extensive studies of

crown gall disease. The overall process of infection is considered to be similar for root mat and crown gall (Veena & Taylor, 2007).

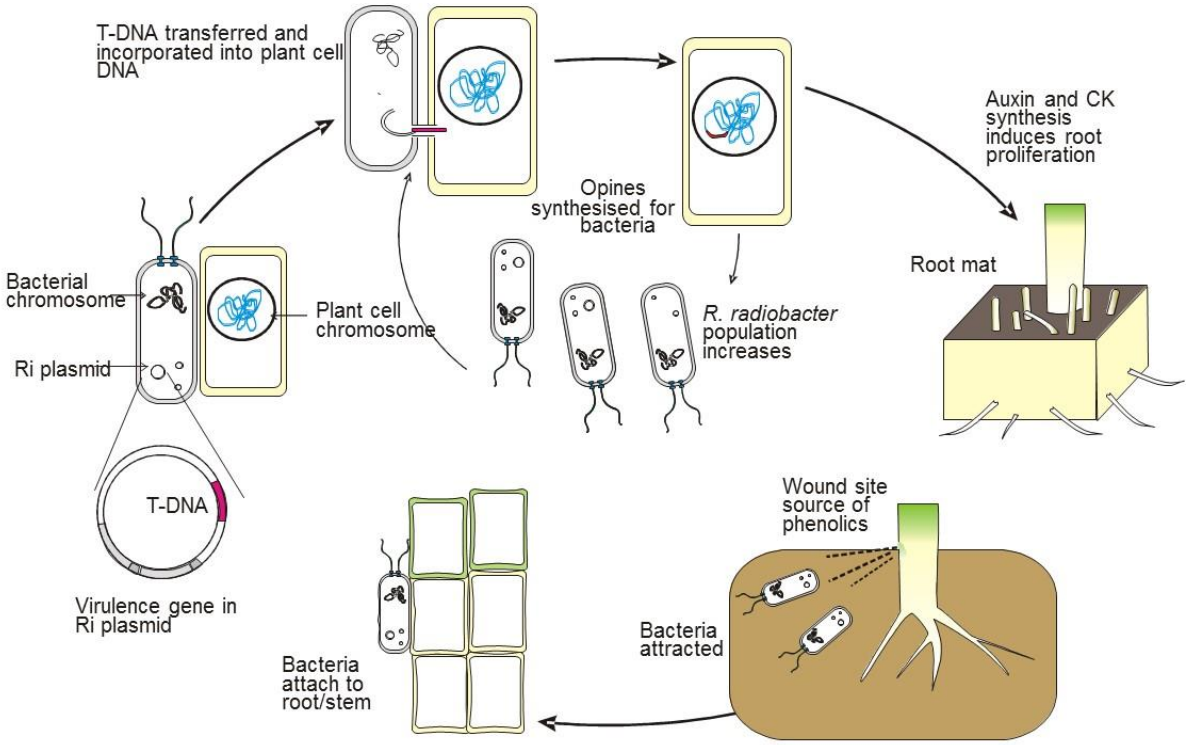

Figure 5. Diagramatic lifecycle of rhizogenic *R. radiobacter*, showing transfer of the T-DNA segment of the pRi plasmid (red) and transformation of plant cells

The development of root mat and crown gall is activated by fresh wounds on roots or stem. Metabolically active wounded plant cells produce exudates containing phenolic compounds that act as signal molecules; bacteria move to the wound site along the chemical gradient and attach to plant cells. Two of the signal molecules, acetosyringone and ∝-hydroxyoceto-syringone, have been shown to activate expression of the virulence gene (*vir*) in *R. radiobacter* (Stachel *et al*., 1985). The signal molecules allow *R. radiobacter* to recognise susceptible plant cells and initiate DNA transfer. With crown gall it has been shown that tumour formation only occurs if plants are inoculated within 2 days of wounding, coincident with the cell division of wound healing (Drummond, 1979). With crown gall in grapevine, it was shown that during the process of wound healing the cambium generates cells that are susceptible to infection and transformation by *A. vitis* (Creasap *et al*., 2005).

The ability of *R. radiobacter* to induce root mat, or crown gall, is dependent on the presence of one or more large plasmids (Van Larebeke *et al*., 1974; Chilton *et al*., 1982; Moore *et al*., 1978). Strains of the bacterium that do not contain the plasmids do not cause root mat or crown gall.

Infection occurs when a piece of the plasmid DNA, known as the transferred DNA (T-DNA), is transferred from the bacterium and incorporated into the host plant nuclear DNA. The T-DNA on both Ri and Ti plasmids is around 10-30 kbp in size (Gelvin, 2003). Genes contained on the Ri plasmid are expressed when inserted into the plant genome leading to a plant hormone imbalance that results in uncontrolled root proliferation at the infestation site (Chilton *et al*., 1982). Root-inducing activity has been shown to be conferred by three *rol* genes on the Ri plasmid (Kiyokawa *et al*., 1994). Two proteins (rolB and rolC) produced by genes *rolB* and *rolC* are reported to be enzymes with auxin-glucosidase and cytokinin-β-glucosidase activities respectively; the function of rolA protein has not been elucidated (Estruch *et al*., 1991a, b). In some cases the major determinant is a hypersensitivity to auxin that produces the excessive proliferation of roots (Stafford, 2000). Morphologically, roots induced by rhizogenic *R. radiobacter* are very similar in structure to wild type roots except that they are longer, more numerous and, most notably, the direction of their growth is not affected by gravity (Veena & Taylor, 2007). With crown gall it has been shown that galls contain abnormally high levels of cytokinins, which promote cell division, and auxins, which stimulate cell enlargement (Drummond, 1979). Plant cells that have been transformed by incorporation of T-DNA from the Ti plasmid are autonomous with regard to production of the plant hormones auxin and cytokinin (Akiyoshi *et al.,* 1984). Similarly, plant cells that have been transformed by incorporation of the Ri plasmid are able to grow in the absence of plant growth regulators (Veena & Taylor, 2007).

Weller *et al*. (2004) showed that the Ri plasmid could potentially be harboured by a number of other bacteria, including members of the genera *Ochrobactrum* and *Sinorhizobium,* which were also able to induce symptoms of root mat in tomato and cucumber. In the related disease crown gall, it has recently been shown that various *Agrobacterium* and *Rhizobium* species are associated with the disease in raspberry, not just a single species (Kusmanovic *et al*., 2015).

Infected plant cells synthesise simple novel metabolites, known as opines, that are not found in normal plant tissues (i.e. an example of natural genetic engineering). The pattern of opines synthesised is determined by the type of virulence plasmid in the bacterium and, in general, the virulence plasmids also confer on the infecting bacterium the ability to utilise the same opines as nutrients (Chilton *et al*., 1982). As noted earlier, strains of rhizogenic *R. radiobacter* can be classified according to the type of opines they produce. The most common strains are agropine-type, mannopine-type, cucumopine-type and mikimopine-type (Veena & Taylor, 2007). A study of the Ri plasmids associated with cucumber and tomato root mat in the UK and France found that the majority of isolates (15 out of 17) produced cucumopine (Weller et al., 2006). The isolates included ones from cucumber affected in the 1970s as well as isolates

obtained subsequently from cucumber and tomato. Two isolates obtained from tomato in 2003 produced an unidentified opine that was non-cucumopine. These isolates were obtained from a tomato nursery with mild symptoms of root mat. In the same year, another tomato nursery within 1 km of the first and which showed severe symptoms of root mat, yielded exclusively cucumopine pRi. It was suggested that the difference in symptom severity between these two nurseries was related to the pRi and not to the chromosomal background. In Japan, the opine produced by Agrobacterium biovar 1 strains associated with hairy root disease of melon, another cucurbit crop, was identified as mikimopine (Moriguchi *et al.,* 2001); this opine is closely related to cucumopine produced by cucumopine pRi.

Rhizogenic *R. radiobacter* strains also differ from each other in terms of polarity of infection. Some strains are capable of inducing root growth only on the apical surface of carrot or beetroot discs (polar types), whereas others induce root proliferation on both apical and basal surfaces (non-polar types) (Ryder *et al*., 1985).

Some of the opines (conjugal opines) secreted by crown gall tumours induce strains of *R. radiobacter* that are donors of the Ti plasmid to produce a diffusible substance, known as the conjugation factor. This conjugation factor enhances the transfer of the Ti plasmid between strains of *R. radiobacter* (Zhang *et al*., 1993).

There is evidence that *R. radiobacter* can move internally within cucumber and tomato plants. The bacterium was recovered from the top of tomato plants inoculated by a root drench, and from the heads of naturally infected cucumber (O'Neill, 2001). In Russia, rhizogenic strains of *R. radiobacter* were isolated from affected roots, cucumber and tomato stems and internal tissues of fruits and seeds (Ignatov *et al*., 2015). In rose plants, the use of antibiotic resistant mutant strains of *A. tumefaciens* conclusively demonstrated migration of the bacterium to points 5 cm below and 25 cm above a puncture inoculation point (Marti *et al*., 1999). The bacterium was isolated from vascular fluids and is presumed to move in the xylem with the transpiration stream.

Recently (Kyndt *et al*., 2015), it was shown that many types of cultivated sweet potato are naturally transgenic food crops. Two different T-DNA regions originating from *Agrobacterium* spp. were detected in many genotypes of sweet potato and the foreign genes were expressed at detectable levels in different tissues of the sweet potato plants. It was suggested that *Agrobacterium* infection of sweet potato occurred in evolutionary time and the T-DNA provided a trait, or traits, that were selected for during domestication around 8,000-10,000 years ago.

7. Factors influencing infection and symptom expression

a) Inoculum concentration

The effect of inoculum concentration was investigated on cucumber plants in PC 149. The number of plants developing symptoms at inoculum concentrations of 10^4, 10^5 and 10^6 cells/ml (5 ml applied per plant), was 3/34, 6/24 and 10/24 respectively; infection at lower concentrations was nil or very low (O'Neill, 2001). In this factorial experiment, there was evidence of greater infection on plants inoculated at 1 or 3 weeks after sowing (9/24 and 8/24 respectively), than at 5 weeks after sowing (3/24). There was no evidence that wounding roots by slitting cubes with a sterile scalpel blade prior to inoculation resulted in increased infection.

In pathogenicity tests with strains of *Agrobacterium*, a concentration of 10^7 cells/ml is commonly used. In a study on the effect of bacterial concentration (2×10^3 to 7×10^9) on transformation of tomato leaf discs by *A. tumefaciens*, transformation was greatest at 5×10^8 cfu/ml and decreased significantly at levels less than 2×10^7 cfu/ml (Davis *et al*., 1991). The three varieties tested differed in their susceptibility to transformation.

b) Plant age

Inoculation studies in cucumber (PC 149) demonstrated that 1, 3 and 5 week old plants were equally susceptible to infection, whereas studies in tomato (PC 241), found that root mat symptoms developed in cv. Claree to the greatest extent when inoculated at 4 weeks after sowing. Plants inoculated at 0, 8 and 12 weeks were also susceptible but to a lesser extent (Table 6).

Table 6. Effect of inoculation timing and isolate on occurrence (%) and severity (0-3) of root mat in tomato cv. Claree – 2008 (PC 241)

Treatment (inoculation timing)	**Month inoculated**	**First symptoms**	**Symptoms on 5 Aug 2008**	
			Isolate MN % plants (severity)	**Isolate WS % plants (severity)**
1. Uninoculated	-	-	0	0
2. At sowing	Dec	4 Jun	20 (0.2)	28 (0.2)
3. 4 weeks after sowing	Jan	15 Apr	100 (1.4)	12 (0.1)
4. 8 weeks after sowing	Feb	21 May	38 (0.4)	0
5. 12 weeks after sowing	Mar	21 May	24 (0.2)	0

Recent work in the Netherlands (Marta Streminska, pers comm.) found that 2 week old tomato seedlings developed symptoms in 2-3 weeks whereas 5-6 week old seedlings took 2-3 months to develop symptoms. These results suggest that infection which results in symptom expression is more likely during propagation and possibly also in the first few weeks after planting out.

With crown gall disease, it is reported that young and actively growing plants are more susceptible to infection and tumor induction (Moore *et al*., 2001).

c) Physical and chemical factors in the root zone

To date there appears to have been few experiments directly investigating effect of root zone physical and chemical environment on tomato root mat.

R. radiobacter is capable of growth in culture up to 35ºC (Young *et al*., 2001), with an optimum of 20-28ºC (Hayward & Waterston, 1965a). Growth of most *Rhizobium* species occurs in the pH range 5-9 with some growing as low as pH 4 and others as high as pH 10.5 (Young *et al*., 2001). Van Kerckhove (2015) reported that *R. radiobacter* isolates from cucumber and tomato grew at temperatures from 4 to 44 °C and pH from 4 to 11. In hydroponic tomato the root zone temperature is usually between 20-30ºC and pH is 5.5-6.5. The tomato root zone temperature and pH therefore appear likely to be conducive to growth of *R. radiobacter*.

With hairy root of apple, 28ºC was optimum for growth of the causal bacterium in culture, while 24-28ºC was optimum for infection.

Moisture level appears to have different effects on infection and symptoms expression. With hairy root of apple, the incidence of infected plants was greatest at a relatively dry 60-75% soil moisture while the greatest root weight occurred at 90% soil moisture (Hildebrandt, 1950). Similarly, in rockwool cucumber, infection was greatest on relatively dry unwrapped (free-draining) rockwool slabs while the greatest extent of root mat occurred in waterlogged slabs where the plastic wrapper was left unslit (O'Neill, 2001). Current practice among growers where slabs are severely affected by root mat is to remove the wrapper to facilitate drainage and thereby reduce the risk of *Pythium* root rot.

A contrary observation occurred with regard to the incidence of root mat in tomato plants on coir slabs with different chip:pith ratios, and presumably different drainage characteristics. The greater incidence of root mat was found in slabs with the lower chip:pith ratio, which were probably wetter than other slabs (see section e below). Biological or other factors may account for the different effects of moisture level for tomato on coir compared with cucumber on rockwool and apple in soil.

The effects of irrigation solution nutrient levels and conductivity on tomato root mat have not been investigated.

The effect of light on tomato root mat has not been investigated.

It has been suggested that oxygen level in irrigation solution may influence root mat infection and/or development. Some Dutch tomato growers are reported to flush standing water from pipework before applying the first irrigation of a day to plants in order to avoid using water with a reduced oxygen content (P. Bouwens, Grodan, pers. comm.). Work in Belgium is investigating whether bacterial slime in pipework influences root mat, possibly through oxygen consumption or possibly through acting as a reservoir of rhizogenic *R. radiobacter*. As yet, there appear to be no conclusive results on whether or not oxygen levels in irrigation solution affect root mat. A direct effect with reduced oxygen increasing *R. radiobacter* population densities appears improbable as the bacterium is aerobic. However, some strains are capable of anaerobic respiration in the presence of nitrate and most strains can grow at reduced oxygen levels (J Elphinstone, pers. comm.). Possibly reduced oxygen may influence root mat indirectly, through an effect on the composition of the microorganism population around roots (e.g. reduced competition), or on root function (e.g. root damage). The Koppert Protocol 2015-2016 for control of root mat recommends dissolved oxygen should be in excess of 7 mg/L and that the drip water for the first irrigation round of the day should be checked every 14 days. It suggests that low levels will inhibit the maintenance of a diverse root microbiology.

d) Variety

Recent work in the Netherlands found common rootstocks such as Maxifort and Emperador were susceptible (Marta Streminska, Wageningen University, pers comm.). In Belgium, the variety Kanavaro was found to be less susceptible than varieties Briosa and Foundation (van Kerckhove, 2015).

e) Growing medium

Root mat has been recorded in the UK in tomato crops grown on rockwool slabs, coir slabs, peat slabs and in NFT (O'Neill, 2001; D Hargreaves, pers. comm.; M Taylor pers. comm.). In a small inoculated trial at ADAS Wolverhampton in 2000, the effect of growing medium on root mat in tomato cv. Espero was examined. One month after sowing and immediately before planting, slabs were inoculated with rhizogenic *R. radiobacter*. At 12 weeks after inoculation, root mat severity was significantly less in coir slabs (0.7 on 0-4 index) than in rockwool (1.9) or peat slabs (1.6) (O'Neill, 2001).

Large differences in the incidence of root mat in tomato plants were recently recorded on three different types of coir slab with different chip/fibre ratios (S. Mayne, ADAS) (Table 7). All plants were on rootstock Optifort and from the same propagator, suggesting an influence of root zone environment. Potentially this may be caused by differences in the root zone environment influencing the population density of rhizogenic *Rhizobium radiobacter* developing around roots, and hence the chance of infection. In recent work in Belgium, controlled inoculation studies on different rockwool and coir growing media also observed differences in the time and severity of tomato root mat, suggesting an influence of root zone environment (Andy Lee, Grodan, pers. comm.).

Table 7. Occurrence of root mat symptoms in tomato plants on Optifort rootstock grown in different formulations of coir substrate – 18 August 2015. All plants originated from the same propagator, and were grown at the same site. 1000 plants assessed per substrate.

Coir slab	**Chip/pith ratio**	**Occurrence of root mat**	
		% cubes affected	% with severe symptoms (> index 3)
Brand 1	50/50	17.6 (1.0)	10.3 (1.91)
Brand 2	40/60	13.6 (1.09)	5.3 (1.92)
Brand 3	70/30	1.5 (0.3)	0.1 (0.74)

( ) – Standard error

f) Wounding

Agrobacterium rhizogenes (*R. radiobacter*) is described as exclusively a wound pathogen (Hayward & Waterston, 1965a). Rhizogenic *R. radiobacter* is believed to compromise the normal wound-healing process by infecting cells that, in the absence of the pathogen, would normally differentiate into functional plant tissue; this is how tumorigenic *A. vitis* initiates crown gall in grapevine (Creasap *et al*., 2005). Host tests are usually done by stab inoculation of the rhizogenic *R. radiobacter* into the plant, so there is a fresh wound created at the time of inoculation. No difference was found when cucumber plants were inoculated by soaking intact roots compared with damaged roots (root tips cut off) in a suspension of rhizogenic *R. radiobacter*, although the number of plants tested was low (O'Neill, 1994). In a factorial trial at STC, inoculum concentration and plant age significantly affected incidence of root mat in cucumber, but not root wounding. In this instance, roots were wounded by making one cut to the full depth of the propagation cube, 4 cm long and 2 cm from the seedling base, with a sterile scalpel blade, immediately before inoculation. In the identification of rhizogenic *Agrobacterium* bv 1 as the cause of root mat in cucumber (Weller *et al*., 2000b) and tomato

(Weller *et al*., 2000a), plants were inoculated after wounding roots with a scalpel blade as described above; undamaged inoculated treatments were not included.

The literature on crown gall clearly demonstrates that tissue wounding is important for infection of plants by tumorigenic *R. radiobacter* (see section 6); one report states that tumour formation only occurs if plants are inoculated within 2 days of wounding, coincident with the cell division of wound healing (Drummond, 1979). Although experiments to date have not demonstrated a requirement for imposed root damage to permit development of root mat in cucumber, it is possible that natural wounds occurring at lateral root emergence, or the experimental procedures used for growing the plants have produced sufficient root damage to allow infection. We found no experimental work on the effect of root damage on the susceptibility of tomato plants to root mat.

Recently, work on walnut indicates that surface contamination of seed with tumorigenic *A. tumefaciens* bv1 can result in crown gall when seed are germinated and grown without imposed wounding (Yakabe *et al*., 2012). Following immersion of bleach-disinfected stratified (4°C) walnut seed in a 3 x 10^7 cfu/ml suspension for 15 h, 94% of seedlings had developed galls when assessed after growing in a glasshouse at 30ºC for 6 months; most galls developed on the main tap root at sites of lateral root emergence. On average, seedlings developed 13.8 galls/seedling. A similar high incidence of infection occurred when uncontaminated seed was sown in potting medium infested with *A. tumefaciens* (2 x 10^7 cfu/g). Additionally, it was shown that all seedlings contained systemic populations of the marked strain of *A. tumefaciens* used to inoculate seed or potting medium, including seedlings without gall symptoms. The bacterium was not detected in negative controls. It was also shown native avirulent strains of *A. tumefaciens* were frequently present in seedlings and that seedlings grown from inoculated seed were less likely to harbour avirulent *A. tumefaciens* than non-inoculated negative control seed.

If tomato seed is unknowingly contaminated with rhizogenic *R. radiobacter*, or the irrigation water or growing medium is contaminated with the bacterium, it seems possible that root mat infection may similarly occur during germination or young plant production in the absence of imposed wounding. As walnut galls were predominantly at the site of lateral root emergence, it seems likely that natural wounds here are sufficient for infection. Treatment of tomato seed with sodium hypochlorite as a precaution against PepMV would reduce any rhizogenic *R. radiobacter* population on the seed but is unlikely to reduce any internal infection. It is also possible that hypochlorite treatment may make the seed more susceptible to infection by removal of non-pathogenic strains of the bacterium, or other competing microorganisms. The period from sowing tomato seed on a propagation nursery to dispatch of young plants is around 5-7 weeks (depending on whether plants are grafted or ungrafted). Due to the

relatively slow development of root mat symptoms, infected plants may show no visible symptoms at the time of plant dispatch from a propagator.

The walnut crown gall study (Yakabe *et al*., 2012) concluded that while management practices reducing man-made wounds may be helpful in reducing root gall formation, it was likely that most wounds which provide *A. tumefaciens* infection courts occur during natural root growth. The majority of galls formed at lateral root initiation sites where tap root tissue had split during secondary root emergence, and around adventitious shoots located around the soil line. It would seem likely that lateral root emergence from main roots, and possibly adventitious roots around the stem base, may provide natural wound sites for tomato root mat infection.

8. Disease spread

Tomato root mat disease does not appear to spread rapidly between plants. It is quite common to find rockwool slabs with one plant severely affected and other plants in the same slab displaying no symptoms. Possibly this is because plants become less susceptible to transformation by the Ri plasmid as they age, or alternatively because pathogen populations are no longer present above threshold levels for infection, especially if the diseased plants were infected and transformed in another location.

Symptoms of tomato root mat disease are rarely, if ever, observed when the new season's plants arrive on a nursery. They are usually first reported in April, around 3-4 months after planting. The change in incidence of plants infected and affected by root mat over a crop's life has not been fully examined. Work in PC 241 examined natural infection in a commercial crop of cv. Jack Hawkins grown on rockwool slabs untreated with microbial amendments. Root mat was first observed on 15 April (3% of plants affected) and increased to affect 12% of slabs by 21 June 2008 (Figure 6). There was no further increase over the remainder of the season, although disease severity increased. Most affected slabs were bounded by unaffected slabs. Groups of affected slabs were more common along a row than across rows, this may reflect spread along a row from slab to slab, or possibly latent (symptomless) infection in a specific tray at planting (McPherson 2009) (Table 8). Under commercial conditions incidence may be expected to be randomly distributed or follow planting patterns – especially if infection is introduced on certain clusters of transplants. Information on the change in incidence of affected plants over time, coupled with tests to determine the proportion of asymptomatic plants carrying the Ri plasmid, could help to determine if there is more than one cycle of infection in a crop each season.

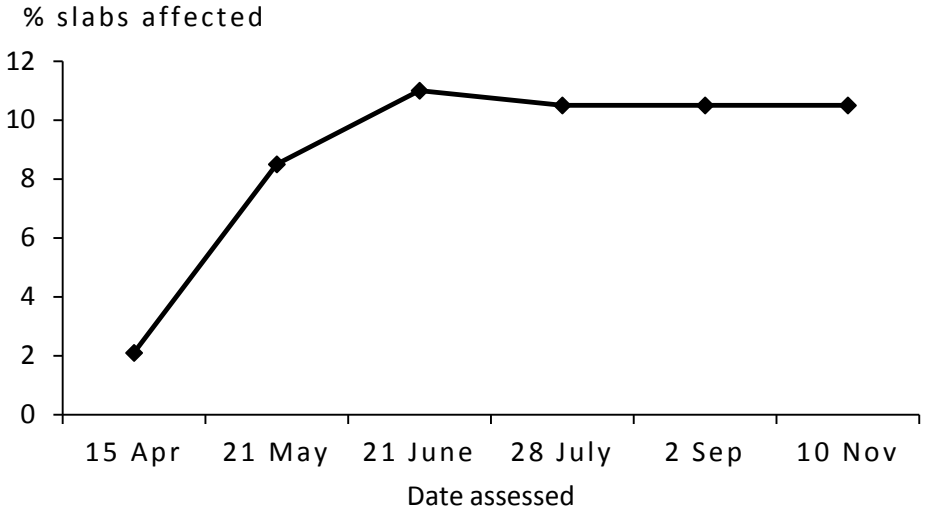

Figure 6. Development of root mat over time in a commercial tomato crop, cv. Jack Hawkins, on rockwool slabs - 2008

Table 8. Grouping of root mat affected tomato plants along and across rows of rockwool slabs – November 2008

Number of affected adjacent slabs in the group	Number of slabs in each category	
	Along rows	Across rows
One	44	89
Two	9	6
Three	2	0
Four	3	0
Five	1	0
Six	1	0

9. Survival of vector bacteria and plasmids

Pathogenic *Agrobacteria* are known to be able to survive for a long time in the absence of a host. In a plot bearing only symptomless weeds, Ti-harbouring strains of *Agrobacterium* were detected in soil 16 years after removal of diseased plants (Krimi *et al*., 2002). Ri strains of *R. radiobacter* have been detected around roots of weeds in soil around UK cucumber glasshouses (Weller *et al*., 2006).

In the absence of plant material, *Rhizobium radiobacter* survived in non-sterile soil for over a year, at a high population for around 100 days. On dry plastic and concrete it survived for 1 day but not 3 days. The bacterium was found to survive in cucumber hydroponic solution for at least 3 weeks (O'Neill, 2001). In sterile distilled water, *Agrobacterium tumefaciens* was

reported to survive and maintain pathogenicity for over 20 years at ambient temperature (Iacobellis & Devay, 1986).

Persistence of root mat is very variable. On at least one tomato nursery the disease has occurred each year from 1999 to 2016, frequently, but not always, at a high incidence. On other tomato nurseries the disease has occurred occasionally at a low incidence and then not been seen again for several years, or has persisted at a low incidence (<1%) for many years. This sporadic nature of root mat disease was also noted in cucumber crops in the 1970s. In tomato the number of UK nurseries affected by the problem has increased over the last few years (Dr Phil Morley pers comm). In Belgium, the proportion of crops affected increased from 8% in 2011 to 26% in 2012 (van Kerckhove, 2015). In 2015 during tomato root sampling work in PC 281b severe root mat was noted on 3 out of 6 UK nurseries visited in July (Sarah Mayne, ADAS).

10. Control

Once a plant is infected with the Ri or TI plasmid, there are no known treatments which will prevent symptom development. Consequently, the current focus for control of both root mat and crown gall disease is prevention of infection. As with other plant diseases, this may be achieved by use of natural host resistance, by environment manipulation to make conditions less favourable for infection or by reduction/elimination of rhizogenic *R. radiobacter* inoculum.

a) Control by host resistance

Many cultivated plant species are not natural hosts for tumorigenic or rhizogenic *R. radiobacter*. The molecular basis for non-host resistance is unknown, although many mechanisms have been suggested (e.g. inefficient integration of T-DNA into the plant genome; *R. radiobacter*-induced programmed cell death) (Escobar & Dandekar, 2003).

In PC 241, aubergine rootstock Madonna was investigated as a potential control for root mat in tomato. At the time of the work (2007), root mat had not been observed in aubergine. Grafted plants of cv. Jack Hawkins on Madonna were planted on rockwool slabs on a nursery with a history of the disease. Although no root mat symptoms developed on the grafted plants, incidence of root mat on ungrafted plants was extremely low so no conclusions could be drawn.

Grower observations indicate that many tomato varieties and rootstocks are susceptible and to date none have been identified as resistant. Recent work in Belgium found that the variety Kanavaro (11% infection) was less susceptible than Briosa (57%) and Foundation (63%) (Van Kerckhove, 2015).

Good cultivar resistance to crown gall has been identified in several plant species. For example, varying levels of crown gall susceptibility have been described in plum, peach, grapevine, aspen and rose. Out of 50 *Rosa* species inoculated with a highly virulent strain of *A. tumefaciens*, 5 were highly resistant, 17 were moderately resistant, 17 were moderately susceptible and 11 were highly susceptible (Zhao *et al*., 2005). The molecular basis for cultivar resistance is not generally known (Escobar & Dandekar, 2003). Crown gall resistance in aspen was found to be negatively correlated with cytokinin sensitivity, suggesting the T-DNA initiated plant hormone synthesis is insufficient to initiate tumours in resistant cultivars. It is possible that further tomato varieties/rootstocks (in addition to Kanavaro) with useful resistance to root mat may be identified or developed.

b) Control by environment manipulation

The primary controllable environmental requirement for the development of crown gall is a plant wound. Careful cultural practices that prevent unnecessary plant wounding can significantly reduce crown gall. Protection from frost damage and control of chewing insects and nematodes can be crucial in preventing natural wounds that can act as sites of natural infection (Burr & Otten, 1999). Timely removal of infected plant material can also prevent continued inoculation of the soil with large populations of pathogenic *R. radiobacter* derived from crown gall tissues (Escobar & Dandekar, 2003).

The effect of plant wounding on the susceptibility of cucumber and tomato to root mat has not been well studied; nor the effect of prompt removal of affected plants.

c) Control by inoculum reduction

Disinfection to reduce inoculum

Attempts to prevent the disease in cucumber initially focussed on sanitation and use of chemical disinfectants to reduce inoculum. Several disinfectant products were fully effective in removing the bacterium from concrete paths and drip pegs. On one cucumber propagation nursery attention was given to rectifying concrete cracks harbouring soil, plants were propagated on new polythene over concrete and a strict hygiene protocol was adopted. Here, ADAS/Fera tests over a number of years showed that the proportion of glasshouse environment samples testing positive for *R. radiobacter* was gradually reduced while those testing positive for Ri plasmid was reduced to zero. In contrast, on a cucumber production nursery, strict hygiene and disinfection of a glasshouse and equipment between crops was largely unsuccessful, likely due to the difficulty in maintaining freedom from infection sources. In cucumber, root mat disease is no longer a significant problem as most nurseries grow three crops a year and the short life of each crop means there is generally insufficient time for severe symptoms to develop.

i) Biocide treatment of surfaces

In previous work on root mat disease, we showed that the following disinfectants resulted in large reductions in *R. radiobacter* populations:

Product	**Active ingredients**
Iodel	iodine
Jet 5	hydrogen peroxide + peracetic acid
Unifect G	glutaraldehyde + QAC
Horticide	glutaraldehyde + QAC
Bleach	sodium hypochlorite

In broth suspension tests, where samples were taken every 30 seconds for 3 minutes following addition of the test disinfectant at a recommended rate, high rate sodium hypochlorite (250 ppm), Horticide, Iodel and Jet 5 were fully effective within 30 seconds, and Glucid and low rate hypochlorite (25 ppm) bleach within 1 minute (Table 9). Menno Florades at 1%, Reciclean at 100 ppm and Sterilite at 1% were ineffective at 3 minutes when sampling ceased.

Table 9. Efficacy of various biocides against *R. radiobacter* as determined by a nutrient broth suspension test at 27°C – 1998 (PC 149)

Disinfectant product	**Rate of use**	**Active ingredients**	**Recovery of *R. radiobacter* after (mins)**					
			0.5	**1**	**1.5**	**2**	**2.5**	**3**
Bleach (Deosan)	250 ppm	sodium hypochlorite	-	-	-	-	-	-
	25 ppm	sodium hypochlorite	+	+	-	-	-	-
Glucid	50 ml/L	glutaraldehyde (20%)	+	+	+	-	-	-
Horticide	1:50	glutaraldehyde + QAA	-	-	-	-	-	-
Iodel	8 ml/L	iodine (2%)	-	-	-	-	-	-
Jet 5	11 ml/L	hydrogen peroxide/PAA	-	-	-	-	-	-
Menno Florades	1:100	organic acids	+	+	+	+	+	+
Reciclean	100 ppm	hydrogen peroxide formic acid	+	+	+	+	+	+
Sterilite tar oil	1:100	phenolic oils	+	+	+	+	+	+
Virkon S	50 g/5 L	potassium persulfate	+	+	+	+	+	+

+ bacterial growth; - no growth.

In tests where a *R. radiobacter* suspension was allowed to dry on concrete, then sprayed with disinfectant and swabbed, no viable *R. radiobacter* was recovered after treatment with Deosan (40 ppm hypochlorite), Horticide (2 % product), Iodel (12.5 % product) or Jet 5 (9 % product).

Subsequent broth suspension tests demonstrated good efficacy with Vitafect (quaternary ammonium compounds) at 1% v/v with no *R. radiobacter* recovered after 30 seconds; the product was not effective at 10 ppm even after 3 mins exposure time.

A practical test was done in which drip pegs taken from a tomato crop affected by root mat were cleaned and disinfected according to the nursery protocol, and then tested in the laboratory for presence of *R. radiobacter*. The nursery treatment was a high pressure water clean of drip line and irrigation pegs hung on crop support wires, followed by spraying with Horticide (2%), followed by a water rinse. *R. radiobacter* was recovered from both untreated peg samples (10 and 1 colony respectively) and not from any of four treated samples.

A further practical test was done to determine the efficacy of steaming once-used rockwool slabs taken from a tomato crop affected by root mat. Slabs were steamed for 5 hours in an adapted shipping container. No *R. radiobacter* was recovered from rockwool samples taken after steaming. However, in associated laboratory work, an experiment indicated that non-rhizogenic *R. radiobacter* added to steamed slabs could acquire Ri plasmid, indicating that the steaming process had not destroyed the plasmid (O'Neill, 2001).

Alternative chemical disinfectants with more persistent activity (e.g. Geosil, new Domestos) and some novel treatments (e.g. chlorine dioxide, electrolysed water and Foamstream heat treatment) with potential for use in disinfection programmes during tomato crop turnaround are now being marketed. Geosil and Foamstream were found to be effective against *Pythium* and *Phytophthora* in root pieces from ornamental plants in recent MOPs work (AHDB Horticulture project CP 124). Geosil and the new Domestos are reported to have components that enhance residual activity. The thicker new Domestos is able to persist longer, maximising possible treatment duration. New Domestos was chosen as unlike other types of bleach this formulation has a defined concentration of sodium hypochlorite rather than a range. The use of the new domestos as a disinfectant was carried out experimentally and cannot be put through the system when plants are present. Information on the efficacy of new products and potential treatments against *R. radiobacter* may enable growers to devise more sustainable disinfection programmes with good activity against the root mat pathogen. No work was done in previous AHDB Horticulture projects on the effect of disinfectants specifically on Ri plasmid survival.

ii) Water treatment

In an experiment exploring potential use of sodium hypochlorite and Jet 5 applied in irrigated water to cucumber plants in propagation for prevention of root mat, although the rates of use examined were safe to cucumber, they were ineffective at the rates and frequency used against *R. radiobacter.* After 30 days, the bacterium was recovered from all plants (McPherson, 2009). More recently in Belgium, studies have examined methods for removing bacterial biofilms in irrigation lines (Van Kerckhove, 2015). A combination of 20 ppm hydrogen peroxide and UV treatment of recycled water was reported to be the most effective treatment.

The effect of slow sand filtration (SSF) in removing *R. radiobacter* (*Agrobacterium tumefaciens*) from water was recently examined in Poland. A water reservoir feeding into an experimental SSF was inoculated with a bacterial suspension and water samples which had flowed through the filter were tested by qPCR (Kubiak *et al*., 2015); SSF efficiency was measured as % reduction of pathogen DNA in water measured before and after filtration. Mean *R. radiobacter* levels were reduced by 81-88%.

In AHDB Horticulture project PC 241, slow sand filtration was examined for possible effects on root mat disease in cucumber and tomato. The work was inconclusive as few symptoms of root mat developed in either inoculated trials at STC (2006 and 2007) or in two trials on commercial tomato nurseries (2008) (McPherson, 2009).

Biocontrol with K84

Rhizobium rhizogenes strain K84, and its improved form K1026, have been used successfully to control crown gall in many plant species (Moore & Warren, 1979; Escobar & Daridekar, 2003). Control is thought to be achieved primarily by a highly specific, anti-agrobacterial antibiotic, called agrocin 84, the synthesis of which is encoded on a plasmid (p AgK84). This antibiotic targets RNA synthetase in tumorigenic *Agrobacterium* strains and inhibits tumour formation. However, the antibiotic is only effective against a subset of pathogenic strains of *A. tumefaciens* that are able to take up the antibiotic. Other antimicrobial substances are also produced.

Several other avirulent *Agrobacterium* strains have been exploited for control of crown gall. In grapevine, the non-tumorgenic *A. vitis* strain F2/5 inhibits crown gall when applied to wounds prior to inoculation with tumorigenic strains. There is evidence that strain F2/5 prevents transformation by inducing necrosis in the cambium, so that cells susceptible to transformation are not generated (Creasap *et al*., 2005).

In 1999, *Agrobacterium radiobacter* K84 (Galltrol) significantly delayed occurrence of root mat symptoms in an inoculated cucumber crop; on re-testing the following season, K84 was again effective, reducing incidence of cucumber root mat at 13 weeks after planting from 94% to 50%, and also reducing disease severity (O'Neill, 2001). Unfortunately, K84 is not registered for use in the UK and efforts in 2009, jointly with Becker Underwood (licence holders), to gain its registration for control of crown gall on nursery stock were unsuccessful. The active ingredient of K84 is considered to be a genetically modified organism by regulatory authorities and currently this prevents registration in the UK.

Other biocontrol agents

There is good reason to believe biological treatments could reduce tomato root mat by influencing the population of rhizogenic bacteria around tomato roots. Specifically, recent work on crown gall disease showed that a quorum-sensing signal is produced by populations of *A. tumefaciens* that controls transfer of the Ti plasmid. Transfer of the Ti plasmid only occurs at high population densities of *A. tumefaciens*, when concentration of the signalling molecule is high. The quorum sensing gene is located on the plasmid. It was also shown that a high concentration of the signalling molecule increased severity of plant symptoms (i.e. number of emerging tumours) (Haudecoeur & Faure, 2015). Assuming quorum sensing also operates with root mat disease, biological products might reduce root mat if they prevent the population of rhizogenic *Rhizobium* around roots from reaching a threshold concentration where plasmid transfer occurs.

Biological control of soilborne diseases typically takes one of two routes: (1) addition of products containing specific microorganism selected for their ability to suppress the target pathogen; (2) addition of complex microbial communities contained in compost or commercially available microbial products. Some examples of both types of product were examined for control of root mat in cucumber and tomato in early AHDB Horticulture-funded work (PC 149). A *Pseudomonas* isolate (3992A) was very effective in a preliminary trial in 1999 but proved ineffective in a replicated trial in 2000. Matured pine bark was also ineffective (O'Neill, 2001). In a follow-on project (PC 241), five biological treatments were tested in 2008 for their effect on root mat.

The products were:

- Biomex SA (a mixture of T*richoderma harzianum, Trichoderma konigii, Trichoderma polysporum* and *Trichoderma viride*)
- Ecoguard GN (*Bacillus licheniformis* SB3086)
- Gliomix (*Gliocladium catenulatum*)

- Rhizopro (*Bacillus subtilis* var. *amyloliquefaciens* FZB24)
- Trianum P (*Trichoderma harzianum* strain T-22)

In an initial study at STC on tomato cv. Claree in 2006, although very few symptoms developed, molecular tests at 8 weeks post-inoculation detected rhizogenic *R. radiobacter* in the roots of all plants except those treated with Gliomix, and the uninoculated control. In a trial on cucumber in 2007, symptoms developed in all treatments except those treated with Gliomix and Biomex SA (Table 10) (McPherson, 2009). Further work on tomato in 2008, was inconclusive as no symptoms of root mat developed in any treatment.

Table 10. Effect of some biological products on root mat in cucumber – 2007 (PC 241). Products applied three times before inoculation with rhizogenic *R. radiobacter* and weekly thereafter.

Treatment	**Rate of use (per 500 ml)**	**Active ingredient**	**Occurrence of root mat symptoms after 16 wks on:**	
			Cube	**Slab**
1. Uninoculated	-	-	-	-
2. Inoculated	-	-	+	-
3. Biomex SA	0.5 ml	*Trichoderma* spp.	-	-
4. FZB	0.25 ml	*Bacillus* spp.	+	+
5. Garshield	50 µl	Garlic extract	+	+
6. Gliomix	1 g	*Gliocladium catenulatum*	-	-
7. GLD	50 µl	Garlic extract + SA derivative	+	+
8. PHC Compete Plus	0.65 g	Various microorganics; yucca extract	+	-
9. Seasol	1.7 ml	Bull kelp concentrate	-	+
10. Stimagro	0.25 g	*Streptomyces* sp.	+	+

In a nursery based observation study, Biomex SA, Gliomix and Trianum P were applied to tomato plants twice in propagation (December) and once post-planting (February). Trianum P appeared to reduce root mat incidence (from 11% to 3.7%), severity and occurrence of plant death whereas Biomex and Gliomix had no obvious effect. In a second study (a replicated experiment on a tomato nursery), Ecoguard GN and Rhizopro were applied 4 times between 19 February and 10 April 2008, before the appearance of any symptoms. However, neither product reduced root mat compared with untreated plants.

In work on crown gall of cherry, *Bacillus subtilis* isolate BCA6, *Pseudomonas fluorescens* isolate BCA11 and *Trichoderma viride* isolate BCA46 all significantly reduced crown gall when applied 24 h before inoculation with *A. tumefaciens.* In a field trial, *B. subtilis* applied as a soil drench just before planting cherry seedlings in a naturally infested soil reduced incidence of infected plants from 11% to 2.4% (Gupta & Khosla, 2007).

Crown gall in grapevine and raspberry was reduced by two *Pseudomonas* species (Khmel *et al*., 1998). In further work on crown gall disease, it was shown that various bacteria producing the enzyme ACC deaminase significantly reduced the development of tumours on tomato plants when roots were soaked in the bacterial strains 4-5 days before injection of stem wounds with pathogenic *Agrobacterium* (Toklikishvili *et al.,* 2010). The effective bacteria were strains of *Pseudomonas putida* and *Burkholderia phytofirmans*. The fresh mass of tumours formed on plants pre-treated with ACCD-producing bacterial strains was typically four to fivefold less than tumours formed on control plants. It was also shown that the protective effect from ACCD-producing bacteria was associated with reduced ethylene production by the tumour mass. A transgenic tomato plant expressing a bacterial ACCD was highly resistant to crown gall formation.

In addition to a direct effect, biological control products may also reduce disease indirectly through activation of host defence mechanisms. In recent work on control of tomato bacterial speck, caused by *Pseudomonas syringae* pv. tomato, it was shown that *Bacillus subtilis* strain QST713 (i.e. Serenade ASO) induced plant defence related genes (Fousia *et al*., 2016).

These results demonstrating reduction of crown gall by various microorganisms, taken together with the report of quorum sensing controlling transfer of Ti plasmid (Haudecoeur & Faure, 2015), suggest there is potential for prevention or reduction of root mat disease in tomato by altering the microbial populations around roots.

It has been proposed that the ratio between non-pathogenic and pathogenic *Agrobacteria* affects incidence and extent of crown gall. To support this theory, it has been shown that more abundant non-pathogenic strains could physically block the infection sites on plants (Lippincott *et al*., 1977).

In grapevine, two non-pathogenic strains of *R. vitis*, isolated from grapevine nursery stock in Japan, denoted VAR03-1 and ARK-1, were demonstrated in field trials to inhibit grapevine crown gall (Kawaguchi, 2013). There was evidence that the two non-pathogenic strains inhibit grapevine crown gall by different mechanisms (Kawaguchi & Inoue, 2012). Strains VAR03-1 and ARK-1 reduced crown gall disease incidence to 24% and 15%, respectively, compared with that in untreated plants. ARK-1 was effective when roots were soaked for one hour in a cell suspension of 2×10^8 cells/ml. This biocontrol strain established in the

rhizosphere and persisted inside grapevine roots for up to 2 years. The non-pathogenic strain VAR03-1 was earlier reported to reduce the frequency of crown gall of grapevine, rose and tomato caused by tumorigenic strains of *A. vitis, A. rhizogenes* and *A. tumefaciens* in greenhouse tests (Kawaguchi *et al*; 2008).

In PC 241, an isolate of rhizogenic *R. radiobacter* obtained from a nursery showing 'weak' symptoms of tomato root mat was examined to determine if it offered protection against a strain that was associated with 'severe' symptoms. In trials in 2006 and 2008, tomato plants were inoculated with the 'weak' strain followed by the 'severe' strain, or just the severe strain. Few symptoms developed in either year so no conclusions could be drawn (McPherson, 2009).

Biocontrol with natural products

Recently a vermicompost amendment to soil was shown to result in population decline of *Agrobacterium tumefaciens* (Strauss *et al.,* 2015). After a 4-week exposure, the *A. tumefaciens* population in some cases declined below detection limits. This suppressive effect appeared to be predominantly biotic as heat-treated vermicompost had no impact on *A. tumefaciens* populations. A positive correlation was found between *A. tumefaciens* suppression and microbial community diversity. In the same study, two commercial microbial fermentation mixtures, marketed as soil amendments, were found to be ineffective in reducing *Agrobacterium* populations.

In 2015, use of Trianum P (*Trichoderma harzianum*) and ProParva (an extract of plant-based materials) was suggested to reduce root mat disease in tomato. Growers have applied these products to the surface of propagation cubes soon after planting/receipt of plants as a preventative measure; and/or on to cubes visibly affected by root mat. We were unable to locate any grower feedback or other evidence to support the use of these treatments for reduction of root mat. Feedback from tomato growers visited by ADAS in July 2015 was that the NatuGro system, involving application of Trianum P, ProParva and ProTerrum (see https://www.koppert.com/resilient-cultivation/the-natugro-system/ for information) was not providing control of the disease. Work is required to examine the efficacy of biological products applied in propagation and after planting to determine if they have a beneficial effect against tomato root mat.

Various plant extracts have been shown to possess antibacterial activity *in vitro*. Both the essential oil and a methanolic extract of *Teucrium pollum* (Lamiaceae) inhibited growth of *R. radiobacter* in culture (Purnavab *et al*., 2015). Major components of the active substances were ∝-pinene myrcene, sinapic acid and eugenol. If biopesticides with these or similar active

ingredients become available in the UK they may warrant consideration for inclusion in products screened for control of root mat.

Selection of products to test

Several biopesticides are now permitted on tomato and others are in the process of registration for use on tomato. Some other biological products do not claim plant protection activity and are considered outside of plant protection product regulations. The following products were examined as candidate test substances in a primary screen of products for efficacy against *R. radiobacter* when applied in propagation (Table 11). It is envisaged that some combinations of products will also be tested. Products considered and excluded are shown in Table 12.

Table 11. Biological products considered for evaluation in a primary screen to determine efficacy in prevention of root mat (PE 029) – 2016

Treatment	**Product**	**a.i.**	**Rate**
1	Trianum P	*Trichoderma harzianum* T-22	Apply 1.5 g in 2.5 L water/m^2
2	ProParva	Plant auxins	1 ml ProParva per m^2 of surface of sowing tray or pots
3	Jet 5	Hydrogen peroxide	40 ppm
4*	Proradix	*Pseudomonas* sp. DSMZ 13134	0.0001g of product per plug (in 1 ml)
5	Serenade ASO	*Bacillus subtilis* QST 713	Apply 1 ml product in 100 ml per m^2 of plugs
6	Carbon Gold	Enriched biochar	Placed in a layer between plug and cube

*Treatment not yet approved

Table 12. Products considered and not selected for primary screen

Product	Active ingredient	Reason for non-inclusion
1. K84 (USA)	Modified *Agrobacterium tumefaciens*	GM. Low prospect of UK registration
2. AgriPhage CMM (Canada)	Bacteriophage of *Clavibacter michiganensis* ssp. *michiganensis*	Likely that activity is specifically against Cmm
3. Potato soft rot bacteriophage (UK; APS Biocontrol)	Bacteriophage of *Pectobacterium* spp.	Activity is specifically against *Pectobacterium* spp.
4. Pro Terrum	Plant-based amino acids and peptides that 'stimulate biological activity around roots'	Preference given to ProParva which is recommended for use in propagation; consider including in nursery trials as part of Koppert NatuGro programme
5. Fulvic 25	Fulvic acid formulation that promotes healthy growth in poor soils and artificial growing media	Consider for use in a second screening experiment
6. Compete Plus	'Dispersible rhizosphere inoculant of beneficial soil microbes'	Consider for use in a second screening experiment
7. T34 Biocontrol	*Trichoderma asperellum* strain T34	Consider for use in a second screening experiment

11. Knowledge gaps

As a result of this review and discussion at the project start-up meeting (23 March 2016) various gaps in knowledge and questions pertinent to the control of tomato root mat were identified. These are summarised below. Additional potential research areas looking at more fundamental aspects of tomato root mat biology are not considered here.

Sources of infection

1. Does rhizogenic *R. radiobacter* occur on commercial batches of tomato seed?
2. Is rhizogenic *R. radiobacter* present in irrigation water or growing media on propagation nurseries? Or associated with sciarid flies or other insects that frequent the tomato root zone?
3. Can the Ri plasmid persist in the environment in the absence of *R. radiobacter* or other vectoring bacteria?
4. Is there latent root mat infection in tomato plants at receipt on production nurseries?

Control by host resistance

5. What is the relative susceptibility to infection of:
    - Seedlings germinating in plugs (propagation nursery)
    - Young plants growing in cubes (propagation nursery)
    - Young plants rooting into slabs (production nursery)
    - Plants well established on slabs (production nursery)?
6. Is there a useful level of resistance to root mat in any tomato genotypes?
7. Can induction of host resistance (Systemic Acquired Resistance or Induced Systemic Resistance) in tomato provide any control of root mat?

Control by inoculum reduction

8. How effective are microorganisms, biological preparations and biocides at maintaining rhizogenic *R. radiobacter* at nil or low population levels in the root zone and the wider glasshouse environment?
9. Does hypochlorite treatment of tomato seed for *Pepino mosaic virus* adequately control any *R. radiobacter* on/in seed?

Control by environment manipulation

10. Can we reduce opine accumulation to deprive *R. radiobacter* of nutrition and prevent population increase?
11. Does handling of plug plants or propagation blocks result in root damage sufficient to significantly influence susceptibility to infection? If so, can handling practices be adapted to minimise root damage and reduce infection?
12. Can we mask/interfere with phenolic compounds produced by tissue wounds and thereby reduce movement of rhizogenic *R. radiobacter* towards susceptible root tissue?

13. Does hypochlorite treatment of tomato seed increase susceptibility to infection by rhizogenic *R. radiobacter* by removal of non-pathogen strains and/or other competing microorganisms?

14. Would application of non-pathogenic microorganisms to seeds soon after hypochlorite seed treatment, especially root colonising bacteria, reduce the susceptibility of young plants to root mat, for example by colonisation of natural wound sites where lateral roots emerge?

15. Does irrigation solution temperature, pH, oxygen level, conductivity, nutrient form or level significantly influence the susceptibility of tomato roots to infection by rhizogenic *R. radiobacter*?

16. Does the water holding capacity of a slab, profile of water distribution in a slab, or irrigation frequency, influence susceptibility of tomato plants to root mat?

17. Do environmental and crop management actions directed at switching plants from generative to vegetative growth increase susceptibility to root mat? Does induction of vegetative growth result in increased lateral root production?

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Glossary

biovar – the name applied to a population distinguished on the basis of biochemical or physiological properties

genomovar – the name applied to strains which are phylogenetically differentiable, but are phenotypically indistinguishable

geotropic – growth of a plant in response to gravity

neoplastic – induces new tissue growth

opines – low molecular weight novel metabolites synthesised in plant tissues following incorporation of plasmid DNA into the plant genome; over 30 different opines have been described. They are amino acid derivatives used almost exclusively by bacteria as a source of carbon and nitrogen.

plasmid – a genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand

quorum sensing – a signalling system between bacteria

rhizogenic – root inducing

T-DNA – transfer DNA; the section of a plasmid transferred into a plant cell and incorporated in the plant genome

tumorigenic – tumour inducing