

Review of the potential for biological control of wild radish (*Raphanus raphanistrum*) based on surveys in the Mediterranean region

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Summary Wild radish (*Raphanus raphanistrum*) (Brassicaceae) is one of southern Australia's worst weeds of cropping. The potential for biological control of this weed in Australia is being investigated in the weed's original distribution, southern Europe and the circum-Mediterranean region. Surveys for insects and pathogens have been made throughout the Mediterranean region, concentrating on southern Portugal, northern Tunisia, the Mediterranean coast of France, and southern Greece. While many of the organisms found are specialists on the family Brassicaceae, most of these are not sufficiently host specific to exclude the risk to canola (*Brassica napus*), the most important crop related to wild radish. Another close relative is the edible radish (*Raphanus sativus*). Wild indigenous populations of edible radish are not known and it has been hypothesised that edible radish has been developed in antiquity from wild radish, even though they are classified as separate species. For this reason, emphasis has been placed on finding biological control agents that attack the seed or reproductive parts of the wild radish or have a host range restricted to wild radish. Agents reducing seed set that are being studied include insects that are highly specialised in their mode of feeding on the plant such as the flower gall fly *Gephyraulus raphanistri*, an insect that has only been recorded from wild radish.

Keywords Wild radish, *Raphanus raphanistrum*, biological control.

INTRODUCTION

Wild radish (*Raphanus raphanistrum* L.) (Brassicaceae) is one of the most important weeds of crops in southern Australia (Poole and Gill 1987). Control of this weed is usually by a combination of herbicide, crop and pasture rotation and management (Cheam and Code 1995). One result of current management has been a rise in herbicide resistance in this weed (Bowran 1998).

The potential for classical biological control of this exotic weed in Australia is being investigated in the weed's original distribution, southern Europe and the circum-Mediterranean region. This review summarises this investigation, outlining the questions that need to be resolved and the prospects for the future.

BIOLOGICAL CONTROL STRATEGY

Classical biological control of wild radish is a difficult objective. In the sections below are summarised the main issues, which are mainly to do with the safety of biological control because of the shared evolution between wild radish and some important crop species.

Genetics and evolution of *Raphanus* Wild radish relatives in the family Brassicaceae comprises about 13 tribes, 375 genera and 3200 species (Hewson 1982, Schulz 1936). The genus *Raphanus* is included in the tribe Brassiceae, which is regarded as a natural grouping. Within the Brassiceae, most phylogenetic evidence points to common ancestry for *Raphanus* and the *Brassica rapa/oleracea* lineage, which also includes canola (Gómez-Campo and Prakash 1999).

Within the genus *Raphanus* there are two species, *R. sativus* L. (edible radish) and *R. raphanistrum*, with three subspecies; *R. r. raphanistrum* (wild radish), *R. r. landra* (Moretti ex DC.) Bonnier & Layens, and *R. r. rostratus* (DC.) Thell., based on the revision of the genus by Pistrick (1987). Some authors of earlier literature list five subspecies. The origins of edible radish are not known. It has been hypothesised that it is derived from one of the subspecies of *R. raphanistrum*, although it is evidently a distinct species (Pistrick 1987). All subspecies of *R. raphanistrum* are found in the Mediterranean region, the most likely centre of evolution of this and related species. The phylogeny and origin of wild radish introduced into Australia is currently being investigated as part of the project based in France.

Australia has 24 species from within the tribe Brassiceae (Hewson 1982). All are introduced (Hewson 1982) and many are weeds. The most important crop in Australia that is closely related to wild radish is canola, *Brassica napus* L., aside from edible radish grown as a vegetable. Wild radish itself has no useful aspects in Australia (Cheam and Code 1995).

Edible radish in Australia The familiar edible radish is grown in market gardens mostly near cities (Burt 1999, Hocking 1997) and is popular for home vegetable production. The production cycle is very short (4–12 weeks) and the plants do not have time to

flower before harvest. Australia grows about 150 ha producing about 1500 tonnes of radish and exports a small quantity to Asia (Murison 1995).

In addition to radish as a vegetable, Asian radish varieties (daikon) are a potential export crop to Korea and Japan for use in pickles (Nguyen 1997). Some varieties of radish can also be used as a short season forage crop or as an oil seed. Radish seed can also be used as sprouts. Presently, these are potential crops in Australia, but the safety of potential crops also needs to be considered in a biological control project.

A biological control strategy Wild radish is an annual, depending on seeds to survive to the following year. A biological control agent has to have a direct or indirect effect on seed production. This can be either by causing a direct or indirect reduction in seed number (eating seeds, killing or stressing the plant so that less seeds are produced), or by indirectly reducing seed quality. Secondly, the agent must be very host specific. It has to be shown that the agent will not attack canola, a crop closely related to the weed. Even so, the morphology of the fruit of *Raphanus* (a silique comprised of segments containing single seeds) is sufficiently different from *Brassica* species (silique without segments), to indicate that maybe there is a different associated fauna.

The situation with edible radish is more problematical. It will be difficult to find potential agents with sufficient specificity and introducing a biological control agent that damages radishes in home gardens will be very unpopular.

This combination of factors points to a strategy of searching for agents that:

1. have very high levels of host specificity and that cause stress to leaves or stems of the plant such that seed quality or quantity declines, or
2. have feeding habits that directly destroy flowers, siliques or seeds, since this part is not commercialised in edible radish.

SEARCH FOR POTENTIAL AGENTS

Surveys in the Mediterranean region Surveys were undertaken in southern parts of Portugal, France, Greece, and northern Tunisia. These surveys started in 1997 and continued each year to 2001, primarily during March, April and May, the months during which wild radish plants bolt, flower and complete seed production. Wild radish does not persist from year to year at each site because of succession of the vegetation community, changes in land use and herbicide applications. Consequently new sites were found each year.

Most plants examined in the Mediterranean region could be classified as *R. r. raphanistrum*. This subspecies corresponds to the wild radish in Australia, and is found in the Mediterranean region in disturbed habitats on acid to neutral soil. A second subspecies was examined during surveys, *R. r. landra*, which is found in coastal regions and along riverbanks in France (Pistrick 1987) on neutral to basic soils.

Canola crops were also examined when found in association with wild radish. Four canola crops were found in Tunisia in 1999, where this crop is a recent introduction, but apparently the culture of canola in subsequent years ceased for economic reasons. No crops of edible radish were found during surveys, possibly because commercial production of this plant is located in more northern parts of Europe. At sites with mature dried siliques at least 100 were collected and then X-rayed to examine for the presence of insect damage to seed. We observed up to 11% destruction of seed in samples from 11 sites from Tunisia. Seed from other areas is still being analysed.

About 50 species of phytophagous insects were found associated with wild radish. Most of these have recorded host ranges that include other Brassicaceae species (e.g. canola, cabbage). The species groups that present the most interest for biological control purposes are listed in Table 1. These species have been chosen because they have a direct impact on seed set (e.g. the sawflies) or potentially contain cryptic species that might have high levels of host specificity (e.g. *Ceutorhynchus* spp.). The highest priority species for further study is the flower gall midge, *Gephyraulax raphanistri* because it is widespread, and only recorded from wild radish (at least in recent literature).

Wild radish flower gall midge The larvae of the gall midge *G. raphanistri* develop inside flowers, which are transformed into galls. Up to 19 larvae can be found in a gall. Many gall forming cecidomyiid larvae live in close association with fungi, but this does not appear to be the case for *G. raphanistri*. Development takes about three weeks and galls are found from April to June, implying that the insect has multiple generations each season. The larvae leave the gall to pupate, probably in the soil at the base of the plant. Adults are fragile and probably do not feed, unless on nectar and pollen. The insect has to survive over summer, then autumn and winter, since there does not appear to be an alternative host. It is not known what stages of the life cycle are involved during this long period.

Raphanus r. landra plants in southern France have been found with every flower galled, but this seems restricted to coastal regions. In Tunisia, galls are present on wild radish plants in wheat fields throughout the

grain-producing region. The insect was found during surveys of Greece and Portugal. The species has a widespread European distribution (Sylvén and Solinas 1987), and is not known as a pest of canola, which is widely grown throughout this region.

Another gall midge, *Contarinia nasturtii* (Kieffer) causes identical galls on wild radish, but this species has a wide host range, and is a pest of canola. We can now recognise the larvae of each species and this has enabled the start of experiments designed to learn more of the biology of these insects, and their interaction. Host range in the field is being assessed by placing flowering canola and edible radish plants in areas where wild radish plants are being attacked by *G. raphanistri*.

Plant pathogens No pathogens were found that directly attack the flowers or pre-dispersal seed. Dispersed seed was not examined because diseases associated with this stage are usually not associated with a particular plant species. Fourteen diseases were collected from wild radish and these are in the process of being identified and tested for phytopathogenicity. Preliminary screening of 11 of these diseases has so far not found pathogens with the required level of host specificity, in that the pathogen either developed on edible radish, canola or both. At this stage, the pathogen with the highest priority for further investigation is *Peronospora parasitica* (Pers. ex Fr.) Fr. (= ? *P. brassicae* f. sp. *raphani* Gäum.) This pathogen was found throughout the region surveyed, early in the growth of wild radish and *R. r. landra*, and seemingly not on neighbouring plants of the Brassicaceae family. Dickinson and Greenhalgh (1977) and Satou and Fukumoto (1996) also indicate host range specificity for some strains of this fungus from edible radish, but further work is needed to clarify the situation for strains from wild radish.

DISCUSSION

Biological control has long been regarded as a too difficult option for weeds in the Brassicaceae. The first investigations on the biological control of Brassicaceae started with Lipa *et al.* (1973) in Poland. However, no Brassicaceae are listed in the world catalogue of agents and their target weeds (Julien and Griffiths 1998) despite this being a large family containing many important weeds. At present, at least four genera of cruciferous weeds are being investigated in biological control projects, mainly for USA (Quimby 2000). This includes the *Lepidium* project that is confronted with the problem of assessing the risk to rare and endangered species in the same genus in North America. This will probably not be a major issue in Australia's project against *Raphanus*, since there are no native species in the entire tribe Brassiceae, although Australia has rare and endangered species from other tribes in the family Brassicaceae (e.g. *Lepidium* spp.) (Hewson 1982).

The gall midge would appear to be an ideal agent for the control of wild radish. It is multivoltine and thus may be able to build-up large numbers during the flowering period of wild radish. It is found in habitats and climates closely resembling that of a large part of crop production in Australia. The main question is the one of safety – will it attack canola? The current tests underway in France are attempting to assess this. Will it attack edible radish? Probably yes, since it is evident that edible and wild radish are very similar species. This may be acceptable in Australia for horticultural production, the only difficulty would be with the production of seed.

Part of the assessment process includes field tests of specificity by the monitoring of plots of wild and edible radish and canola. The phylogeny of radish species and subspecies is being investigated because this will provide a framework for understanding the level of specificity of potential agents and for predicting

Table 1. Insect species found attacking wild radish (*Raphanus raphanistrum raphanistrum*) in the Mediterranean region that warrant assessment for use in classical biological control.

Family	Common name	Genera or species	Biology and part of plant attacked
Cecidomyiidae (Diptera)	Gall midge	<i>Gephyraulus raphanistri</i> (Kieffer)	Larvae feed inside flowers causing the formation of a gall, preventing seed, multivoltine
Tenthredinidae (Hymenoptera)	Sawfly	<i>Elinora</i> spp.	Caterpillar-like larvae feed externally on developing siliques and flowers, probably univoltine
Urodontidae (Coleoptera)	Seed-weevils	<i>Urodon</i> spp.	Larvae develop inside single seeds, adult biology unknown, probably pollen feeders, rare
Curculionidae (Coleoptera)	Weevils	<i>Ceutorhynchus</i> spp.	Larvae feed inside various parts of the plant, adults on leaves causing minor damage, probably univoltine

the evolution of these organisms. It is also likely that phylogeny and species identification at the molecular level will be required for any potential agents.

In conclusion, this is a difficult project, but not without hope. Impact studies currently underway suggest that wild radish suffers heavily from damage by natural enemies in its native habitat (J. Vitou and J.K. Scott, unpublished data). A short list of potential agents is under investigation. However, in all cases, any potential agent will require considerable investigation to ensure that it is absolutely safe to be released in Australia.

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