Japanese Beetle Potential Host Range in California

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Japanese Beetle (*Popillia japonica* Newman) is a species introduced into North America that has spread widely throughout the eastern United States. In this area, infestations have proven extremely damaging to horticultural and agricultural plants. Yearly costs for management and mitigation of damage are estimated at US$500 million (USDA 2007). In addition, areas infested with Japanese beetle can be quarantined by trading partners, resulting in losses to farmers and nursery growers mounting into the millions of dollars per year (National Plant Board 2007; Smith et al. 1996). Japanese Beetle is not yet fully established in California, but recent detections in Sacramento County have raised concerns and triggered control activities by agencies tasked with protecting plants from pests.

Japanese beetles have a broad range of host plants. The list of plants used as hosts by Japanese beetle contains more than 300 species (Potter & Held 2002). Because the focus has been on Japanese beetle attacks in gardens and lawns, the host list is dominated by garden plants. Most of the information on host susceptibility to Japanese beetle originated from a landmark survey summarized by Fleming (1972). This rating system was based on written and oral accounts of Japanese beetle feeding damage noted from 1920 through 1963 primarily in the New England area (Fleming 1972).

Although poorly documented, there is little doubt that Japanese beetle can attack many other species of plants not included on the host list (Miller et al., 2001; Potter & Held 2002). This is demonstrated by the sheer diversity of plants that Japanese beetles currently are known to attack. Many of the most common garden plants in California are not grown in the northeastern United States; these plants never would have been noted as hosts based on plants grown in New England. Known hosts span many different plant families, but certain plant groups seem particularly susceptible to attack from Japanese beetle. In addition, certain characteristics such as flower paleness flower color, purple leaves, and release of stress chemicals can promote Japanese beetle attack (Loughrin et al., 1995; Keathley et al., 1999; Rowe et al., 2002; Held 2004; Held & Potter 2004).

In inferring which plants of California are likely to be attacked by Japanese beetle, it is useful to consider the Centrifugal Phylogenetic Method. This method guides choices of potential host plants to test in host specificity studies of insects proposed as biological control of invasive plants. The Centrifugal Phylogenetic Method was explicitly described by Wapshere (1974). It states that if an insect attacks a certain species of plant, the chance of another plant being attacked by the same insect is positively correlated with the phylogenetic (evolutionary) relatedness of the 2 plant species. The more closely related are the plants, the more similar will be their morphology and chemistry; therefore, they are more likely to be recognized as a potential host by particular herbivores or predators. Evidence on the evolution of insect-plant associations supports this view (Futuyma 2000). In addition, many host plant tests for potential biological control agents have demonstrated that non-target feeding tends to

be concentrated in plants related to target hosts (Pemberton 2000; M. Pitcairn, pers. comm.).

At the time it was proposed the centrifugal phylogenetic method was hampered by the limited information available regarding the phylogenetic relationships of many plant groups. Therefore traditional taxonomy was used as a surrogate for explicit phylogenetic hypotheses. The development of phylogenetic systematics and the plethora of DNA sequence data that has become available in the last 30 years has revolutionized our understanding of plant phylogeny (Mishler 2000; Simpson 2010). This increase of knowledge has revealed that much traditional knowledge about plant relationships was quite correct, but some groups were artificial (e.g., Olstead et al. 2001; Xiang et al 2002; Kelch 2003). These finer scale and more accurate hypotheses concerning plant relationships can be used to predict further potential hosts in testing (Briese 2003; Kelch & McClay 2004).

In the case of a polyphagous insect such as Japanese Beetle, we have evidence that it can attack many species. This supplies us with more evidence for inferring other plants likely to be hosts for these species. Using character optimization (e.g., Donoghue 1989), we can predict that those species falling within a lineage defined by 3 or more related taxa are at increased risk of attack from Japanese Beetle. Some of patterns are noted below.

Members of the rose family (Rosaceae) are commonly attacked by Japanese Beetle. Roses (*Rosa* spp.) are preferred hosts, but several other genera are attacked as well (e.g., cherries [*Prunus*], spirea [*Spiraea*], & bramble [*Rubus*]). Related common garden plants in CA include false hawthorn (*Rhaphiolepis*), loquat (*Eriobotrya*), cotoneaster (*Cotoneaster*), and photinia (*Photinia*).

The mallow family (Malvaceae) has many members that are attacked by Japanese Beetle. Related common garden plants in CA include flowering maple (*Abutilon*), flannel bush (*Fremontodendron*), cow itch tree (*Lagunaria*), & lavender star-flower (*Grewia*).

A range of grasses (Poaceae) are attacked by Japanese Beetle, especially in its larval stage. Related common garden plants in CA include Mexican feather grass (Nasella) and fescue (*Festuca*).

Several genera of the knotweed family (Polygonaceae) are attacked by Japanese Beetle. Related common garden plants in CA include wild buckwheat (*Eriogonum*) as well as ornamental knotweeds (*Persicaria*).

Many elms are attacked by Japanese Beetle. Related common garden plants in CA include zelkova (*Zelkova*) & hackberry (*Celtis*).

The exceedingly large daisy family (Asteraceae) contains several known hosts of Japanese Beetle such as sunflower (*Helianthus annuus*) and horseweed (*Erigeron*

canadensis). Related common garden plants in CA include bush daisy (*Euryops*) and boneseed (*Osteospermum*).

Fleming (1972) also includes a list of plants that are not known to be attacked by Japanese beetle. Judging whether relatives of such plants would prove to be safe from Japanese Beetle attack is speculative. This is especially true as many plant families that have species that are not attacked also contain species that are attacked by Japanese beetle. Nevertheless, based on the approach described above one can postulate that some plants are most likely at a lower risk level than plant species related to known hosts.

Conifers include many woody plants that are not known to be attacked by Japanese maple (Fleming 1972). Such genera of Pinaceae as pines (*Pinus*), fir (*Abies*), and spruce (*Picea*) contain non-host species. Cupressaceae genera that contain non-host species include white-cedars (*Chamaecyparis*). Yews (Taxus) are not attacked. Nevertheless, deciduous conifers such as larch (*Larix*) and bald-cypress (*Taxodium*) are attacked. Therefore, dawn redwood (*Metasequoia*) might be attacked as well. Some evergreen Cupressaceae such as arbore vitae (*Thuja*) and some junipers (*Juniperus*) are attacked. Therefore, most PInaceae except deciduous species might be predicted to be resistant to attack, but Cupressaceae would be unpredictable. The genus *Podocarpus* (Podocarpaceae) is reported as a potential host.

Evergreen hollies (Ilex: Aquifoliaceae) are reported as resistant to attack. Only the deciduous winterberry (Ilex verticillata) is reported as attacked by Japanese beetle.

Within the dogbane family (Apocynaceae), the evergreen small periwinkle (*Vinca minor*) is reported as resistant to attack from Japanese beetle, but the deciduous dogbane (*Apocynum*) and milkweeds (*Asclepias*) are susceptible to attack. If this pattern holds, the evergreen large periwinkle (*Vinca major*), common in CA gardens and near old home sites, may be resistant to attack from Japanese beetle.

There are both evergreen (*Euonymus*) and deciduous (*Celastrus*) members of the Celastraceae listed as resistant to attack from Japanese beetle. As this family is consistently resistant, the common in CA genera euonymus (*Euonymus)* and mayten (*Maytenus*) would be expected to be low risk for attack from Japanese beetle.

Two genera of the small family Buxaceae, box (*Buxus*) and pachysandra (*Pachysandra*) are resistant to attack from Japanese beetle. Genera common in CA gardens such as *box* and sweet box (*Sarcococca*) would be considered lower risk for attack from Japanese beetle, based on the centrifugal phylogenetic method.

Japanese Beetles attack several genera of the legume family (Fabaceae), including the clovers (*Trifolium* spp.) and black locust (*Robinia*). However, certain genera of Fabaceae resistant to Japanese Beetles attack are also present in CA gardens. These resistant plants include mimosa (*Albizzia*), sweet-pea (*Lathyrus*), and redbud (*Cercis*). Plants of this family that are common garden plants in CA include brooms (*Cytisus* &

Genista), lupine (*Lupinus*), and acacia (*Acacia*). Inclusion in the legume family (Fabaceae) is a poor predictor of host status for Japanese beetle.

The dogwood genus (*Cornus*) is listed as resistant to attack from Japanese beetle, but the related sweet gum (*Nyssa*) is attacked. Therefore, inclusion in the dogwood family (Cornaceae) is a poor predictor of host status for Japanese beetle.

Several deciduous genera of the olive family (Oleaceae) are known to be resistant to Japanese Beetles attack. These include forsythia (*Forsythia*), ash (*Fraxinus*), and lilac (*Syringa*). At least 2 species of deciduous privet showed feeding from Japanese beetle: the CA privet (*Ligustrum ovalifolium*) and the common privet (*L. vulgare*). It is hard to predict whether the evergreen species grown commonly in CA such as shining privet (*L. lucidum*) and chinese privet (*L. sinense*) would be prone to attack of not. Olive (*Olea*) is also a commonly grown evergreen plant in the olive family. Its host status is unknown.

The laurel family follows a common pattern in which deciduous genera are reported as hosts (i.e., *Sassafras* and *Lindera*), while the evergreen camphor trees (*Cinnamomum*) are resistant to Japanese Beetle attack. If this pattern holds, then bay laurel (Laurus), California bay (*Umbellularia)*, and avocado (*Persea*) could be considered low risk for Japanese Beetles attack.

The examples cited above are not meant to comprise an exhaustive list, but are simply some obvious examples of plants common in California gardens that, based on their close relationships, are at high or low risk for attack by Japanese maple.

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Appendix I:
These plants had no record of Japanese Beetle feeding as of 1972 (based on Fleming 1972)

Acer rubrum - Red maple
Acer saccharinum - Silver maple
Celosia argentea cristata - Cockscomb
Vinca minor - Ground myrtle
Ilex aquifolium - English holly
Ilex cornuta - Chinese holly
Ilex crenata - Japanese holly
Ilex opaca - American holly
Impatiens balsamina - Garden balsam
Begonia rex-cultorum - Begonia
Myosotis sylvatica - Forget-me-not
Buxus sempervirens - Boxwood
Pachysandra terminalis - Japanese spurge
Calycanthus floridus - Carolina allspice
Lonicera fragrantissima - Winter honeysuckle
Symphoricarpos albus - Snowberry
Symphoricarpos orbiculatus - coralberry
Dianthus barbatus - Sweet william
Dianthus caryophyllus - Carnation
Gypsophila paniculata - Baby breath
Gypsophila repens - Creeping gyposophila
Stellaria media - Common chickweed
Celastrus scandens - American bittersweet
Eunonymus alatus - Winged euonymus
Euonymus fortunei - Climbing euonymus
Tradescantia fluminensis - Wandering-Jew
Ageratum conyzoides - Ageratum
Caladium bicolor - Caladium
Centaurea cineraria - Dusty miller
Centaurea cyanus - Cornflower
Centaurea montana - Mountain-bluet
Coreopsis lanceolata - Lance coreopsis
Rudbeckia hirta - Brown-eyed susan
Rudbeckia lacinata - Coneflower
Rudbeckia laciniata hortensis - Goldenglow
Cornus florida - Flowering dogwood
Sedum spectabile - Showy sedum
Chamaecyparis lawsoniana - Lawson cedar
Chamaecyparis obtusa - Hinoki cypress
Chamaecyparis pisifera - Sawara cypress
Chamaecyparis thyoides - Atlantic white cedar
Kalmia latifolia - Mountain laurel
Rhododendron nudiflorum - Pinxterbloom
Dicentra formosa - Pacific bleedingheart
Bambusa vulgaris - Common bamboo
Dactylis glomerata - Orchardgrass
Hedeoma pulegioides - American pennyroyal
Hyssops officinalis - Hyssop
Mentha spicata - Spearmint
Physostegia virginiana - False dragonhead
Albizzia julibrissin - Mimosa
Baptisia australis - Blue false-indigo
Cercis chinensis - Chinese redbud
Lathyrus odoratus - Sweetpea
Convallaria majalis - Lily of the valley
Erythronium albidum - Dogtooth violet
Lilium longiflorum - Easter lily
Lilium speciosum - Showy lily
Lilium tigrinum - Tiger lily
Yucca flamentosa - Adam's needle yucca
Liriodendron tulipifera - Tulip tree
Magnolia grandiflora - Southern magnolia
Magnolia soulangeana - Saucer magnolia
Magnolia virginiana - Laurel magnolia
Nymphaea odorata - American waterlily
Forsythia intermedia - Border forsythia
Forsythia suspensa - Weeping forsythia
Fraxinus americana - White ash
Fraxinus pennsylvanica - Red ash
Syringa persica - Persian lilac
Syringa vulgaris - Common lilac
Papaver nudicaule - Iceland poppy
Papaver orientale - Oriental poppy
Phytolacca americana - Common pokeberry
Abies concolor - Balsam fir
Picea abies - Norway spruce
Picea orientalis - Oriental spruce
Pinus sylvestris - Scotch pine
Pseudotsuga taxifolia - Douglas fir
Tsuga canadensis - Hemlock

Portulaca grandiflora - Common portulaca
Aquilegia canadensis - American columbine
Aquilegia vulgaris - European columbine
Clematis heracleaefolia - Tube clematis
Delphinium formosum - Hardy larkspur
Helleborus niger - Christmas rose
Ranunculus acris - Meadow buttercup
Ranunculus sceleratus - Bitter buttercup
Reseda odorata - Common mignonette
Populus alba - White poplar
Populus alba pyramidalis - Bolleana poplar
Heuchera sanguinea - coralbells
Hydrangea arborescens - Smooth hydrangea
Hydrangea paniculata - Panicle hydrangea
Philadelphus coronarius - Mock orange
Ribes grossularia - European gooseberry
Ribes oxyacanthoides - Northern gooseberry
Antirrhinum majus - Snapdragon
Chelone glabra - White turtlehead
Digitalis purpurea - Foxglove
Verbascum thapsus - Mullein
Vernonica officinalis - Speedwell
Staphylea trifolia - American bladdernut
Taxus baccata - English yew
Taxus canadensis - Canada yew
Taxus cuspidata - Japanese yew
Tropaeolum majus - Garden nasturtium
Callicarpa dichotoma - Beautyberry
Lantana camara - Lantana
Viola odorata - Sweet violet
Viola papilionacea - Butterfly violet
Viola tricolor - Pansy

Putting the phylogeny into the centrifugal phylogenetic method

Dean G. Kelch[1] and Alec McClay[2]

Summary

Phylogeny has long been recognised as an important concept in constructing test plant lists for use in host specificity testing. In the absence of detailed, explicit phylogenies, taxonomic classifications have often been used as a framework for test plant selection. Unfortunately, traditional taxonomies often do not reflect phylogenies accurately. These inaccuracies can take several forms; some taxonomic classifications represent unnatural (polyphyletic) groups. However, the most common instances reflect paraphyletic classifications, in which some, but not all, descendents of a most recent common ancestor are grouped taxonomically. Explicit phylogenies, many based on comparison of DNA sequences, are becoming available for many plant groups. The Internet is an excellent guide to these phylogenies, as one can find references to peer-reviewed articles, abstracts, and unpublished research information. Explicit phylogenies should provide a basis for test plant choice, but the process will also be informed by such guideline criteria as economic importance, regulatory interest, geographic proximity, and ecological similarity. Common phylogenetic patterns include those in which the target taxon is equally distantly/closely related to plant species of concern. This scenario indicates a broad, equally distributed choice of exemplar taxa for host specificity testing. If the target taxon is more closely related to some taxa of concern than it is to others, then a graduated sampling strategy is indicated. Some specific examples are discussed that illustrate these common outcomes.

***Keywords*:** biocontrol, phylogenetic method, phylogeny, weeds.

Introduction

In assessing potential agents for the biological control of weeds, food plant specificity studies play a vital role. Because these studies entail assessing the potential agent's response to a significant number of plant species or cultivars, they utilize a considerable portion of the time and money allotted for agent development. Therefore, one must use criteria for choosing test plants that are both efficient and effective in evaluating potential agents.

There are several major criteria that are used in choosing test plants for food plant specificity studies including propinquity, relationship, and importance. Propinquity refers to the occurrence of the test plant within the release region. Generally speaking, a broad interpretation of release area is preferred, as the vagility of the potential agent is rarely known. In addition, once an agent is approved for release, there are rarely significant regulations preventing the release of an agent throughout the country (see Nechols 2000, Louda & O'Brien 2001). In the case of large countries such as the US and Australia, this can lead to spreading the agent throughout an entire continent. Degree of relationship is an important criterion for choosing test plants, as there are strong correlations between agent host range and taxonomy. Importance of the plant chosen for testing has traditionally applied to agricultural and important range species, but can equally apply to ecologically important plants, as well as rare or endangered species.

The ideal test plant would fulfil all three criteria mentioned above. Nevertheless, the most important criterion is degree of relationship. It is the close relatives of the target weed that are most likely to share the critical features that allow an agent to feed and breed successfully (e.g. chemistry, morphology, and phenology). Ideal biocontrol agents should be monophagous (restricted to the target plant) or, if

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oligophagous (restricted to the target plant and a few related plants), then the non-target plants should not occur in the release area and/or they should be troublesome weeds themselves. It is the close relatives to the target weed that will provide the acid test of monophagy.

Wapshere (1974) emphasized phylogenetic (evolutionary) relationships as the most important criterion for choosing plants to test (although he also acknowledged non-phylogenetic criteria). He called this the centrifugal phylogenetic method. At the time, explicit phylogenies, that is to say detailed hypotheses of evolutionary relationships among organisms, were rare. However, as taxonomy was viewed as being based primarily on phylogeny, it was recommended that the hierarchical taxonomic groupings be used as a guide to test plant choice.

Phylogeny versus taxonomy

The field of systematics has undergone major changes in the last 30 years, the most profound of which is the development of phylogenetic systematics. This field attempts to reconstruct the evolutionary tree of life. A huge body of literature has been generated on the theoretical bases and practical methods for inferring evolutionary relationships and constructing phylogenies. Putative relationships are illustrated by using branching diagrams or trees. Although interpreting these trees quickly entails some preparation, they are powerful cognitive tools. In addition, the development of comparatively inexpensive and rapid methods for sequencing genes and other molecular markers has resulted in a source of new data for use in phylogenetic analysis. By comparing the sequences of particular genes across taxa, systematists have been achieving great insights into the structure of the tree of life. What study after study has found is that traditional taxonomy is not necessarily a pure reflection of phylogeny. Although artificial (polyphyletic) groups are rare in land plants, many taxonomic groups are paraphyletic. This can create problems for planning food plant specificity studies.

Polyphyletic groups are composed of organisms not closely related to each other. They generally are associated on the basis of convergent evolution. Traditional taxonomists were, by and large, able to avoid polyphyletic taxa by comparing whole suites of morphological characters. Polyphyletic groups are more likely to result from single character taxonomy, which was occasionally practised, but more often criticized, by systematists. Nevertheless, when few characters were available to distinguish taxa, polyphyletic groups sometimes resulted. The evidence from DNA sequence data often allows us to identify these unnatural groupings. At the family level, Cornaceae (dogwood family) *sensu lato* (s.l.) and Saxifragaceae (saxifrage family) s.l. are two examples of polyphyletic groupings. In Cornaceae, the small starry flowers with inferior ovaries resulted in dogwoods and the related *Davidia* (dove tree) being classified with the unrelated (to Cornaceae and to each other) *Aucuba* and *Corokia* (see Bremer *et al.* 2002, Xiang *et al.* 2002) (Fig. 1). The herbaceous Saxifragaceae *sensu stricto*, mainly based on the possession of a bicarpellate ovary, traditionally has been associated with several woody taxa such as *Ribes* (gooseberries), *Hydrangea*, *Philadelphus* (mock orange), *Argophyllum* and *Brexia* (Fig. 1). Current evidence indicates that, of these, only *Ribes* is closely related to Saxifragaceae (Savolainen *et al*. 2000, Soltis *et al.* 2000, Soltis *et al.* 2001). The traditional family Scrophulariaceae, which contains a number of weedy genera such as *Linaria*, *Verbascum*, *Veronica*, *Plantago* and *Striga*, has been shown to be composed of at least five distinct monophyletic groups (Olmstead *et al.* 2001).

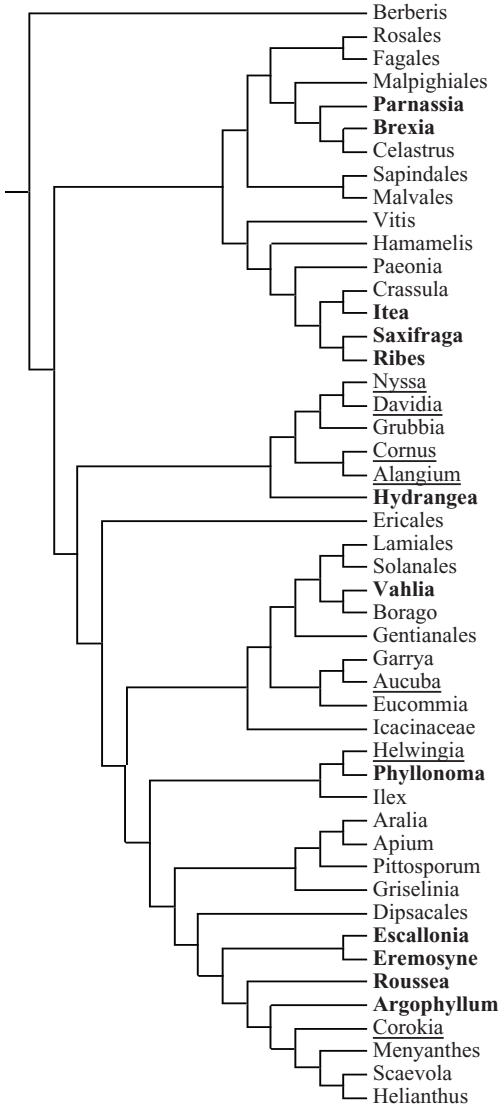

Figure 1. Phylogeny of eudicots showing polyphyly of Cornaceae and Saxifragaceae. Taxa marked in bold represent taxa traditionally placed in Cornaceae. Taxa underlined represent taxa traditionally placed in Saxifragaceae. Adapted from references in text.

At the generic level, recent evidence indicates that the large, woody, legume genus *Acacia* is polyphyletic. *Acacia* subgenus *Acacia*, consisting of about 160 species of pantropical trees and shrubs, is not closely related to subgenera *Phyllodineae* and. *Aculeiferum* (Miller & Bayer 2001, 2003, Robinson & Harrison 2000). *Acacia* subgenus *Phyllodineae* comprises about 960 species from Australasia. In this group are found several economically important species, including some that are noxious weeds in warm temperate and subtropical areas. Unless there is a successful petition to change the type of the genus *Acacia*, the large subgenus *Phyllodinaeae* will become the genus *Racosperma* C. Martius (Maslin *et al.* 2003). As more evidence becomes available, other polyphyletic genera are likely to be identified, especially in large families with poorly differentiated genera (e.g. Compositae, Umbelliferae, and Cruciferae).

Paraphyletic taxonomic groups include some, but not all, of the descendents of a most recent common ancestor. Usually, allied taxa not included within the paraphyletic group display one or more characteristics that make them seem significantly different from their close relatives. Paraphyly is very common in modern taxonomic systems, because, traditionally, paraphyly was ignored or implicitly accepted by systematists.

A classic example of a paraphyletic group is the Pongidae (great ape family), that are paraphyletic to the Hominidae (human family). Based on both morphological and molecular evidence, chimpanzees are more closely related to humans than they are to other great apes (Goodman *et al.* 1998). Therefore, the Pongidae include some, but not all, descendents of the most recent common ancestor of chimps and orangutans. In a purely hypothetical example, if chimpanzees were to become an agricultural pest, one would be in error if one tested potential biological control agents only against members of the Pongidae. Using phylogeny as a guide, such agents, even if narrowly oligophagous, would be much more likely to attack humans than other great apes.

In a plant group that includes weedy taxa, a good example of paraphyly is found in the genus *Brassica* (mustards), which is paraphyletic to *Raphanus* (radishes) (Fig. 2). These genera are distinguished by their fruit morphology; *Brassica* has the typical siliques of the Cruciferae, while *Raphanus* has indehiscent fruits that break into one-seeded sections. Evidence from DNA sequence data shows that *Raphanus* evolved from within the genus *Brassica* (Yang *et al.* 1998, 1999). Both of these groups include weedy strains as well as important food cultivars. Any food plant preference studies carried out within this group should include test plants chosen based on phylogenetic relationships rather than taxonomic grouping. Other paraphyletic plant groups include *Arabis* and Chenopodiaceae. *Lepidium* (Cruciferae) is paraphyletic to the weedy genus *Cardaria* (Mummenhoff 2001). Chenopodiaceae is paraphyletic to the family Amaranthaceae (Downie 1998); this has led to the proposal to unite the two families under the older name Chenopodiaceae.

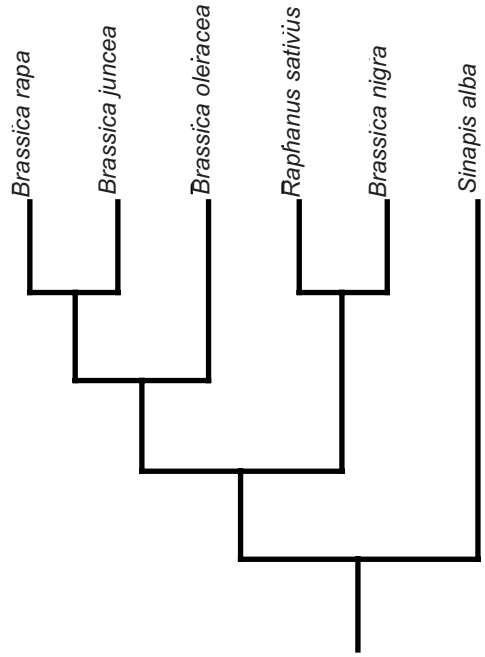

Figure 2. Paraphyly of *Brassica* in regard to *Raphanus*. Phylogenetic tree based on Yang *et al.* (1999).

Phylogeny and biocontrol

Briese *et al.* (2002), in their study of potential biocontrol agents of *Onopordum* (scotch thistle), illustrated how a phylogeny, even if it is not fully resolved, can be used in guiding test plant choice. Briese *et al.* (2002) numbered the clades (groups) or nodes on a simplified phylogeny of the Compositae based on degree of relationship. Theoretically, those taxa in clade 1 would be more heavily sampled than those in clade 2, but not in clade 1. At the distant level of 4 and 5, no sampling was deemed necessary. The authors point out that using this information allows one to save time and money by excluding distantly related plants, even if they are classified in the same family.

Hypericum perforatum (St John's wort), native to Europe, is a noxious range weed in large areas of western North America. *Hypericum* is a large genus of 350–400 species; in North America alone, there are about 60 taxa of *Hypericum*. In the literature on the biocontrol of *H. perforatum*, there are accounts of differential feeding by potential agents on species within the genus. As there are too many taxa to test them all, a phylogenetic framework would be invaluable in choosing the most critical *Hypericum* species for inclusion in food plant specificity studies. Preliminary results of a molecular systematic study (Park & Kim 2001) indicate that *H. perforatum* is more closely

related to some native North American species of *Hypericum* than it is to others. *Hypericum* section *Hypericum*, which contains *H. perforatum* as well as some North American species (e.g. *H. concinnum*) is paraphyletic. Thus, there is evidence from the phylogenetic structure within *Hypericum* that differential sampling within the genus would be indicated in studies of potential biocontrol agents of *H. perforatum*.

The genus *Cirsium* (true thistles) represents an interesting problem in North America. There are introduced species that are noxious weeds (e.g. *C. arvense* and *C. vulgare*), as well as over 90 native taxa, some of which are critically endangered. The traditional taxonomy implies that infrageneric groups (sections) are distributed in both the Old and New Worlds (Petrak 1917). However, a preliminary phylogeny of the genus (Kelch & Baldwin 2003) indicates that the North American native taxa form a clade separate from all Old World *Cirsium* (Fig. 3). Therefore, all North American *Cirsium* are equally distantly related to any Old World species. This result calls for an even sampling of North American taxa in food plant specificity studies.

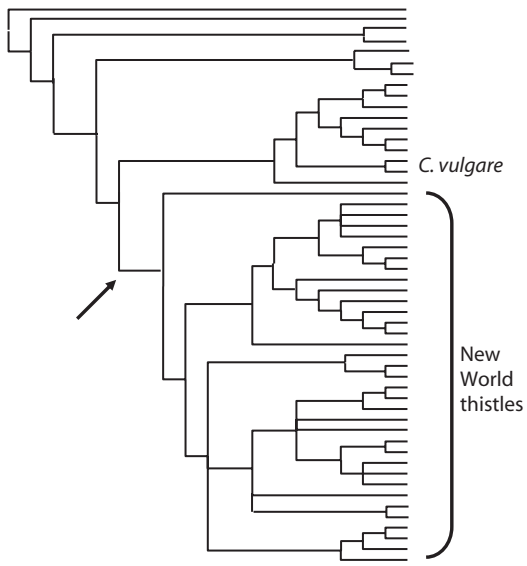

Figure 3. Phylogeny of *Cirsium* (true thistles) indicating New World Clade (arrow) and placement of weedy taxa *C. arvense* and *C. vulgare*. Based on Kelch and Baldwin (2003).

Conclusions

If a well-resolved phylogeny is available, we can use phylogenetic inference to avoid wasting resources on superfluous sampling. In a hypothetical example, two sampled taxa are attacked by the potential biocontrol agent. It is quite likely that all members of the clade representing all descendents of the most recent common ancestor of the two attacked taxa are potential food plants for the agent being studied. Based on the evidence indicated, one cannot rule out that there will be feeding beyond the clade of interest. Nevertheless, this information allows one to concentrate sampling on those untested taxa most likely to be attacked by the potential agent. Note that phylogenetic inference allows information from studies from other geographic regions to inform test plant choice in another region. In addition, inferring the potential pool of vulnerable plant taxa based on limited sampling allows early rejection of candidate agents that show feeding patterns that are too broad for desirable biocontrol agents.

Criteria other than degree of relationship are important in test plant choice as well. These include economic importance, rare or endangered species, ecologically important species, and species of particular concern to government agencies (e.g. wetland species in the US). Nevertheless, all of these secondary criteria should be considered within a phylogenetic framework. Sampling distantly related taxa is a waste of time and resources (Pemberton 2000, Briese 2003).

Much information on plant phylogeny is available on the Internet, but as yet there is no central repository of information. A search should start on the Treebase website <http://www.treebase.org/treebase/>, which is meant as a source for information on the phylogeny of all life. However, as submission of information is voluntary, the results of many studies do not appear in this database. Many journals demand that authors of manuscripts including nucleotide sequences submit all such sequences to Genbank <http://www.ncbi.nlm.nih.gov/>. Multiple sequences for the particular gene in a specific plant group generally indicates a phylogenetic study published or in press. Most entries also include information regarding the purpose of the research that generated the sequence, as well as any pertinent publications. Primary literature database services such as Biosis <http://www.biosis.org>, Web of Science <http://wos.mimas.ac.uk/>, and Agricola <http://www.nal. usda.gov/ag98/> are excellent sources of citations of phylogenetic studies. General Internet search engines such as Google <www.google.com> often can provide information on plant phylogeny. Many professional scientific societies post abstracts of their meetings and many scientists have professional webpages that cite their research interests. These can be a useful source of information on publications and/or addresses of potentially informative personnel.

Acknowledgements

We thank the Wyoming Biocontrol Weed Group, the USDA National Biological Control Institute, and the Lawrence R. Heckard Fund of the Jepson Herbarium at UC Berkeley for providing funding to D.G.K. for this research.

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Ann. appl. Biol. (1974), 77, 201–211
Printed in Great Britain

A strategy for evaluating the safety of organisms for biological weed control

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(*Accepted* 3 *December* 1973)

SUMMARY

A strategy for establishing the specificity and safety of an organism as a biological weed control agent is described. A critical first step is to expose to its attack a small group of plants very closely related and exhibiting morphological and biochemical similarities to the weed. To prevent an erroneous negative result tests are also made on selected cultivated plants, including those closely related to the weed, those of which the associated insects and fungi are little known, those that have evolved apart from or been little exposed to the agent, those attacked by closely related organisms and those already recorded as hosts.

The circumstances under which the strategy might fail to indicate safety are discussed, i.e. polyphagous organisms attacking plants irregularly distributed throughout many families, organisms highly specific to two alternate hosts, and those attacking two or three phylogenetically widely separated plant groups. The additional crop plant testing, included in the overall strategy to deal with such possible failures, is discussed.

It is shown that the strategy would have included *Sesamum indicum* in the list of plants challenged by the bug *Teleonemia scrupulosa* in biological testing for control of *Lantana camara*, thereby forewarning of the attack that was subsequently observed in Africa.

INTRODUCTION

In selecting agents for the biological control of weeds, it is important to demonstrate that the organisms selected are sufficiently specific to the weed that, on introduction to a new country, no damage will be done to plants of economic importance.

Recently Wapshere (1974) compared the various methods used to date and noted that they consisted of two main types:

(*a*) the crop testing method where a large range of crop plants are exposed to the organism.

(*b*) The biologically relevant method proposed by Harris & Zwölfer (1968) examines the biology, specificity and evolutionary relationships between the insect and its host plant and so renders much crop plant testing redundant.

Wapshere (1974) suggested a testing strategy, essentially based on the phylogenetic relationships of the weed, combining some of the safety of crop plant testing with the

relevance of more biologically meaningful methods. In this paper the author examines the scientific foundations of this strategy, discusses situations where it might be unsatisfactory and considers appropriate safeguards against possible failures.

THE PROPOSED STRATEGY

The strategy has two main components:

(A) A centrifugal phylogenetic testing method which involves exposing to the organism a sequence of plants from those most closely related to the weed species, progressing to successively more and more distantly related plants until the host range has been adequately circumscribed. The theoretical sequence for testing the host range of the organisms attacking *Chondrilla juncea* L. is given in Table 1.

Table 1. *Centrifugal phylogenetic method applied to* Chondrilla juncea

Testing sequence	Plants to be tested	Host range determined if plants at that phylogenetic level remain unattacked
1	Other forms of *C. juncea*	Specific to *C. juncea* clone
2	Other *Chondrilla* species	Specific to *C. juncea*
3	Other members of tribe Crepidinae	Specific to genus *Chondrilla*
4	Other members of subfamily Cichoriaceae	Specific to tribe Crepidinae
5	Other members of family Compositae	Specific to subfamily Cichoriaceae
6	Other members of the Order Synantherales. Member of Campanulaceae, Lobeliaceae, etc.	Specific to family Compositae

(B) As a safeguard against failure of the centrifugal method cultivated plants selected by the following five criteria should be tested.

Criterion (i). Cultivated plants botanically related to the weed. This is a natural extension of the phylogenetic principle to include those cultivated plants considered to be at greatest risk. It depends on well-established knowledge of the phylogeny of the flowering plants (Emberger, 1960; Engler, 1964).

Criterion (ii). Cultivated plants for which there is little or no entomological or mycological information. This criterion accepts that, on many occasions, scientific knowledge is inadequate to permit a complete biologically relevant selection of test plants.

Criterion (iii). Cultivated plants which have evolved apart from, or for geographic or climatic reasons have not been extensively exposed to the organisms under study. This criterion reflects the uncertainty, from whatever cause, of the behaviour of phytophagous organisms faced with a new plant species.

Criterion (iv). Cultivated plants known to be attacked by organisms closely related to the biological control agent under investigation. This criterion is so highly dependent on existing knowledge that, at the present time, it can only be used to select plants to test well-known insects and fungi native to Europe and North America. When the

data are available it is a useful additional criterion but is not as essential for safety as the other criteria (Harris & Zwölfer, 1968). Knowledge of the plants attacked by related organisms also indicates whether a genus or other natural group of phytophagous organisms is closely associated with a natural plant group. If there is good evidence of the co-evolution of the phytophages and their hosts further confirmation of host restriction is obtained.

Criterion (v). Any plants on which the organism has previously been recorded. It is clearly necessary to test such plants not least to confirm the veracity of the records.

Harris & Zwölfer (1968) suggested selecting test plants using Criteria (i), (iv) and (v) while (ii) and (iii) are the additional criteria proposed by Wapshere (1974).

SCIENTIFIC BASIS FOR THE PHYLOGENETIC TESTING METHOD

Before proceeding further, it should be noted that potential biological control agents are not by any means all of those attacking a given weed but only those that are, at first sight, sufficiently specific and sufficiently damaging. Throughout the discussion below, therefore, it should be remembered that the great majority of polyphagous organisms associated with a weed have already been excluded, the candidates having been pre-selected to some extent for apparent specificity.

Control of specificity

Specificity is controlled by a series of behavioural and physiological reactions to stimuli governing the feeding and oviposition behaviour of insects and mites and the penetration and subsequent mycelial development of fungi. In some insects a series of stimulatory, inhibitory and deterrent substances and structures controls host range via characteristic behaviour patterns (Thorsteinson, 1960; Beck, 1965, Jermy, 1966, De Wilde & Schoonhoven, 1969). In some cases, as in the reaction of *Pieris* sp. to mustard oil glucosides (Verschaffelt, 1910, David & Gardiner, 1966) and *Chrysolina brunsvicensis* Gr. to hypericin (Rees, in De Wilde & Schoonhoven, 1969), the specificity is largely determined by the presence of one or more token substances. In others, tactile reactions are also involved, e.g. the spines on thistles and burrs stimulating the oviposition of trypetid flies (Currie, 1932; Zwölfer, 1969). In other cases, which include the gall makers (Mani, 1964) and the rust fungi, the organisms react to a complex of chemical substances and have very intimate biochemical relationships with their hosts. In most cases specificity is determined by the improbability that other, particularly less closely related, plants will have the same pattern and concentration of constituents, especially certain amino acids, sugars, enzymes, plant hormones and secondary plant substances (Beck, 1965).

Morphological and biochemical similarity

The testing of organisms by plant phylogenetic methods is based on the assumption that related plants are morphologically and biochemically more similar than unrelated plants. That closely related plants are morphologically similar is a *sine qua non* since plant taxonomy and phylogenetic relationships have been based on such morphological

relationships. That closely related plants are biochemically similar is abundantly documented in Swain (1963) for acetylenic, phenolic, alkaloid and aliphatic compounds.

This does not mean that particular biochemical substances do not sometimes occur widely, but rather that closely related plants are more likely to have particular substances or combinations of substances in common. Thus Kjaer (in Swain, 1963) reports that thioglucosides (mustard oil glucosides) occur in all members of the three closely related families, Cruciferae, Capparidaceae and Resedaceae (all Rhoeadales) so far tested and that they occur only occasionally in a few other families. This morphological and biochemical similarity between closely related plants applies whether or not a single chemical substance or morphological feature triggers feeding or other behaviour or whether the organism depends on a biochemical complex and subsequent growth reaction, as do the gall insects. It applies whatever the pathway by which specificity has evolved, although co-evolution of a phytophagous group and host plant group is likely to have strongly reinforced the reactions to similar morphological and biochemical features of closely related plants.

Testing the critical plant species and a range of related plants

The above discussion suggests that the most critical challenge to the presupposed specificity of the organism is to expose to it the plant species most closely related to its normal host. If this critical test plant remains unattacked or uninfested, there is a strong initial presumption of specificity. If, however, it is attacked or infested, then the next most closely related species should be exposed and this process continued until the host range has been determined.

In practice, because of the possibility of a disjunct distribution of token substances and because closely related species may differ widely in morphological features such as hairiness and cuticular thickness, it is necessary to test against a series of plants. The series should represent a range from closely to less closely related members of the same genus, to members of the same sub-family and eventually to less closely related members of the same or closely related plant families. In this way it is likely that most disjunct distributions of features will be taken into account. This was the procedure followed in testing the *Chondrilla* organisms (Wapshere, 1973).

With the emphasis on crop plants, this phylogenetic approach is extended as Criterion (i), i.e. the exposure to the organism of a range of cultivated plants phylogenetically related to the weed. Where the weed belongs to an isolated family, only members of that family need be exposed (e.g. Casuarinaceae or Proteaceae, Emberger, 1960). Where, however, the weed belongs to a family with close relations, then all crop plants from the related families should be tested. For example, if a *Reseda* species were under investigation, it could be necessary to expose not only members of the Resedaceae, but also all cultivated plants of the superfamily Rhoeadales (i.e. Cruciferae, Capparidaceae, Papaveraceae, etc.). Similarly, if a boraginaceous weed is to be controlled, then all cultivated members of the superfamily Tubiflorales should be exposed.

Assuming that the remainder of the testing programme, i.e. exposure to plants selected on Criteria (i) to (v) has been negative, the phylogenetic testing would lead to one of two distinct conclusions:

First, considerable doubt would be cast on an organism's specificity if it attacked a wide range of plants in the same family or if within a family there were an inexplicable choice of plants that was unrelated to taxonomic relationship with the weed. In this latter circumstance, testing of other crop plants would be necessary.

Secondly, there would be considerable confidence in the specificity of a phytophagous organism if during the phylogenetic testing it attacked only the weed species or closely related members of the same genus. Even if the organism attacked plants in related genera there would be a strong presumption of specificity if there was greater attack on the weed than on related plants (Harris, 1963, 1964).

In both these cases the organism could be sufficiently specific for use in biological control if none of the plants attacked was, or belonged to, a genus containing crop plants.

Nevertheless, there could be circumstances in which although the phylogenetic testing method indicated a high degree of specificity other unrelated cultivated plants were seriously at risk. If the method is to have general applicability these contingencies have to be avoided.

CIRCUMSTANCES IN WHICH THE PHYLOGENETIC METHOD MIGHT ERRONEOUSLY INDICATE SPECIFICITY

Possible examples are as follows:

1. A polyphagous organism attacking a series of plants in different families without relationship to plant phylogeny would reflect lack of feeding inhibitors in certain unrelated plants.

2. Where organisms (e.g. various aphid species and rusts of Gramineae) have two alternate hosts which are not related, specificity to each can be extremely high.

3. An organism which attacks a genus or several related genera in one family and a similar group of plants in another widely separated family due, for example, to their possession of a common feeding stimulant.

In all these cases unrelated plants would be attacked by the potential biological control agent.

It seems extremely unlikely that polyphagous organisms of the type considered at the beginning of this section would not have attacked some of the series of related cultivated plants chosen by Criterion (i). Almost certainly the host range demonstrated during phylogenetic testing would have been wide, and distinct phylogenetic relationships in host range would not have been demonstrated. This case has already been discussed and if such an organism were still to be considered, it would be necessary to test crop plants other than those already selected on Criteria (i) to (v).

The second case does not present as great a problem as at first appears. Organisms such as the rusts and the aphids are specifically adapted to their alternate hosts. If all the developmental stages of an organism from a group containing members with a strict alternation of hosts occur on one host plant, the rust or aphid is obviously monoecious and there is no further problem. However, if one or more stages are absent biological studies and surveys must be made to determine whether the organism is truly monoecious. The missing stage may no longer exist, e.g. *Xanthium* rust, *Puccinia xanthii*, which only exists in the teleutostage (Arthur, 1934), or there may be

an unknown alternate host. In the latter case the developmental stage expected to produce the stage on the alternate host should again be tested against a group of cultivated plants under appropriate climatic conditions, since there is no other method of determining the organism's safety.

Greater problems are posed by those organisms with a disjunct non-phylogenetic host distribution (3). Such disjunct distributions are due to the erratic occurrence throughout various plant families of token feeding or oviposition substances or inhibitors. This problem will therefore be limited to those insects, e.g. many Lepidoptera and certain Coleoptera, which are dependent on token chemical substances to inhibit or initiate and ensure sustained feeding or oviposition. This problem is particularly acute if the critical host behaviour is governed by a yes/no reaction to one particular substance.

The first possibility to be considered is that the feeding or oviposition reaction is a result of a complex of responses to various token substances within a total behavioural sequence depending on a series of reactions to morphological and chemical features of the normal host (Beck, 1965). If, during biological studies, such behaviour is shown to occur, then it is extremely unlikely that another group of plants would have the appropriate chemical and morphological features to serve as host. If a limited host range were indicated by phylogenetic testing and there were no attack on plants selected by Criteria (i) to (v), the organism should be safe to introduce without further testing.

If, on the other hand, a single behavioural or chemical token is the main operative feature in host selection there are two possibilities:

(i) The chemical or behavioural tokens are widespread chemical substances or frequently occurring morphological features. In this case the phylogenetic method would demonstrate a wide host range with no obvious phylogenetic relationship to the normal host. Before introduction, such organisms would have to be thoroughly evaluated on the basis of their performance against all crop plants used in the testing programme (Criteria (i) to (v)). This situation is exemplified by *Pieris brassicae* L. which readily attacks most Cruciferae and other members of the superfamily Rhoeadales and other unrelated plants (e.g. *Tropaeolum*) known to contain mustard-oil glucosides (Verschaffelt, 1910; David & Gardiner, 1966).

(ii) The occurrence of the chemical or morphological token is restricted to a single plant group. Under these conditions phylogenetic testing would have demonstrated a restricted host range centred about the normal host and within one or several closely related genera.

Where the token is a distinctive morphological structure like the spiny fruits of *Xanthium* or the spiny globular seed heads of thistles which serve as oviposition tokens for trypetid flies (Currie, 1932; Zwölfer, 1969) only the results of tests against crop plants with similar structure need be considered to confirm safety.

Where, however, the token is a chemical substance like hypericin, which occurs in certain *Hypericum* species and appears to serve as a stimulant for *Chrysolina brunsvicensis* (Rees, in De Wilde & Schoonhoven, 1969), it is not possible to be certain whether the token exists in other crop plants. In this case, because of the strong specificity suggested by the phylogenetic testing a negative result from testing by criteria (i) to (v) might be considered sufficient to indicate safety for introduction.

TWO TEST CASES

In Uganda it was found (Davies & Greathead, 1967; Greathead, 1972) that *Teleonemia scrupulosa* Stäl., an insect introduced to control *Lantana camara* L. (Verbenaceae), was attacking sesame, *Sesamum indicum* L. *T. scrupulosa* had been introduced earlier to Hawaii, Fiji and Australia (Holloway, in DeBach, 1964) being considered adequately specific to *Lantana*. The question arises whether or not, according to the author's strategy, *S. indicum* would have been selected for testing of *T. scrupulosa* before introduction into East Africa.

Selection would have been made on three criteria: on close relationship (Criterion (i)) since sesame is a cultivated plant of the family Pedaliaciae which like *Lantana* (Verbenaceae) is included in the plant superfamily Tubiflorales (Emberger, 1960; Engler, 1964); on Criterion (ii) because *T. scrupulosa* is a native of Mexico, a region where the entomological knowledge of crop plants is very limited, and on Criterion (iii) because *Sesamum* is a native of Africa and India and has only recently been introduced as a major crop plant into Mexico where *T. scrupulosa* is native.

There would therefore have been strong reasons for testing *T. scrupulosa* against sesame under the author's strategy. Furthermore, it is likely that the phylogenetic testing would have demonstrated an unsuspectedly wide range for *T. scrupulosa*. In the laboratory it is already known to feed on *Tectona grandis* L. among other Verbenaceae, and for this reason it was not introduced into India (Gardner, 1944).

Another test case is provided by the specificity of the flea beetle *Longitarsus jacobaeae* Waterhouse to species of *Senecio* closely related to *S. jacobaea* L. (Frick, 1970).

Frick based his testing on that recommended by Harris & Zwölfer (1968) and limited it almost completely to various Compositae, including a large number of *Senecio* species and a group in the same subfamily, Senecioneae. The plants of other families were chosen on Harris & Zwölfer's criteria as follows: *Helianthemum nummularium* (L.) Mill (Cistaceae) because of a reported occurrence of *Longitarsus jacobaeae* on this plant; three species of Boraginaceae and the legume, *Crotalaria*, because they possess alkaloids similar to those of *S. jacobaea*; *Daucus carota* L. because like *S. jacobaea* it is a tap rooted biennial. No other cultivated plants were tested although a literature survey was made to discover whether *L. jacobaeae* had been recorded on any cultivated plants. Frick did not test any plants attacked by related organisms. Using the present author's strategy, the various *Senecio* species would have been selected during phylogenetic testing, and the various related cultivated Compositae would have been selected on Criterion (i). Almost no plants would have been chosen either because of lack of entomological knowledge (Criterion (ii)) or because they had not been exposed to the organism (Criterion (iii)), since the entomological knowledge of European crop plants is so complete and since all but a few of the major crops in North America have been grown for a long time in Europe, where *Longitarsus jacobaeae* is native. One other plant, *Linum usitatissimum* L. (flax), would have been selected on Criterion (iv) because it is the only cultivated plant attacked by a *Longitarsus* sp. (Bonnemaison, 1962). Therefore, except for the six plants of other families and *L. usitatissimum*, Frick's (1970) selection of test plants was exactly that chosen by the present author's

strategy. Using his selection, Frick concluded that the restriction of *Longitarsus jacobaeae* to *Senecio* and the closely related genus *Emilia* had been clearly demonstrated.

DISCUSSION

To date two main methods have been used to demonstrate the safety of organisms for biological weed control.

(*a*) In the crop testing method each important crop plant is exposed to the organism and if no damage ensues the organism is considered to be safe. However, as Harris & Zwölfer (1968) point out, a negative result does not ensure that plants related to the crop plant are not at risk. The method reveals no biological reasons for specificity nor does it indicate the possible host range of the organism.

(*b*) The series of biologically relevant investigations proposed by Harris & Zwölfer (1968) are more likely to reveal the basis of specificity and indicate the host range of the organism. Unfortunately, it was found that most of their proposed investigations, except those involving plant testing were inapplicable to the demonstration of the safety of the organisms attacking *Chondrilla juncea*. For instance, Harris & Zwölfer (1968) proposed an investigation into the insect's biology with attention to any adaptation likely to restrict host range. There were no readily discernible physiological, morphological or ecological adaptations limiting the gall midge *Cystiphora schmidti* (Rübs.), or two aphids *Dactynotus chondrillae* Nev. and *Chondrillobium blattnyi* Pint., to *Chondrilla*.

Although not insects, to which Harris & Zwölfer restrict their remarks, the rust fungus, *Puccinia chondrillina* Bubak et Syd., and the eriophyid gall mite, *Aceria chondrillae* Can., also had no readily discernible restrictive adaptations. The four *Chondrilla* root feeding insects, the tortricid *Oporopsamma wertheimsteini* Reb., the phyticid *Bradyrrhoa gilveolella* Tr., the buprestid *Sphenoptera foveola* Gebl. and the coccid *Neomargarodes chondrillae* Arch., do indeed use the latex produced by *Chondrilla* to construct larval cocoons or as a protection (Rudakova, 1932; Emelyanova, Pravdin, Kuzina & Lisitzuina, 1932; Kozulina & Rudakova, 1932). But as all Cichoraceae produce a similar latex, the use of this physiological feature does not explain these insects' restriction to *Chondrilla*. The literature review proposed by Harris & Zwölfer indicated that only two of the *Chondrilla* organisms belonged to insect genera with hosts restricted to natural plant groups: the aphid genus *Dactynotus* is confined, except for one record, to Compositae and Campanulaceae, and the cecidomyid genus *Cystiphora* is recorded only on the composite subfamily, Cichoriaceae. The other insects either belong to genera with a large number of unrelated hosts (*Sphenoptera* and *Neomargarodes*) or the hosts of the other species are unknown (*Oporopsamma* and *Bradyrrhoa*). The aphid, *C. blattnyi* belongs to a monotypic genus. The rust genus *Puccinia* and the Eriophyid genus *Aceria* also have species attacking many unrelated hosts (Wapshere, 1974).

Harris & Zwölfer (1968) also suggested that the basis of host recognition should be determined. Unfortunately, there is no readily detectable chemical substance or morphological structure serving as a token stimulant for the *Chondrilla* organisms.

In any case, even if a chemical feeding token is discovered, its occurrence in other plants may not be known and would be difficult to establish.

It will be clear from this discussion and previous comments that none of the methods for demonstrating the safety of biological control organisms for weeds are generally applicable and all have disadvantages.

The strategy proposed by the present author is effective in separating somewhat polyphagous organisms from the monophagous or oligophagous ones which should be used for biological control. It is particularly effective in demonstrating the safety of organisms, such as rusts and gall-makers, which have a close biochemical relationship with their plant host. It is also effective in demonstrating the specificity of those insects whose host selection is based on a complicated behavioural pattern involving many chemical substances or morphological features as feeding and oviposition tokens. After limited additional crop plant testing it can be used to evaluate the safety of organisms having a simple yes/no reaction to a particular morphological feature. In all these cases the number of crop plants to be tested would be considerably less than currently required for organisms from North America and Europe, because application of Criteria (ii) and (iii) would result in the selection of only a few cultivated plants.

With organisms native to other parts of the world a larger number of cultivated plants would be selected on Criteria (ii) and (iii). However, although it would not achieve the desired economy of labour, it would not be a disadvantage if as many plant species as are normally required or more were tested since a greater knowledge of host range and specificity would be obtained, and there would be more certainty that related crop plants would remain unattacked. The strategy is less effective for those insects which are dependent on a single token substance of unknown distribution.

The phylogenetic approach is not effective for organisms which are dependent on widespread morphological features or chemical substances as behavioural tokens, nor for those which seemingly have an unknown alternate host. In both these cases considerable attention has to be given to the additional range of crop plants tested.

The full set of investigations proposed by Harris & Zwölfer (1968) is at least as time consuming and expensive as the crop-testing method and the phylogenetic strategy. However, they have the advantage of yielding a more detailed understanding of host specificity than the crop method, thus providing greater confidence in the final conclusion.

Unfortunately, Harris & Zwölfer's series of investigations were designed entirely with insects in mind and for the most part with insects whose host selection involves fairly specific and readily identifiable behavioural tokens. The crop method and the phylogenetic strategy can be used with all insects as well as with other organisms. Both were used effectively to demonstrate the safety of the *Chondrilla* rust, *Puccinia chondrillina* (Hasan, 1972).

In conclusion, the phylogenetic testing strategy can be used to evaluate safety of organisms for biological weed control without or with some crop testing. Such a combination avoids the disadvantages of the simple crop testing method and those of the investigations proposed by Harris & Zwölfer (1968), while combining the advantages of these methods.

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