

Successful Establishment of Exotic Agents for Classical Biological Control of Invasive Weeds in Virginia

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Abstract

Recent research emphasis in the biological control of invasive weeds in Virginia focused on the following weeds: *Carduus thoermeri* (= *nutans*) (musk thistle), *Carduus acanthoides* (plumeless thistle), *Cirsium arvense* (Canada thistle), *Centaurea maculosa* (spotted knapweed), and *Lythrum salicaria* (purple loosestrife). Besides being effective in the control of musk thistle, *T. horridus* also successfully controls plumeless thistle. Field nurseries of musk thistle were established for the propagation, collection, and subsequent release of *Trichosirocalus horridus* at other sites where they are not established. The rust fungus, *Puccinia carduorum*, has also become established on musk thistle. *P. carduorum* has spread rapidly since its release on musk thistle in Montgomery Co. in southwestern Virginia. Studies on the impact of *Cassida rubiginosa* on Canada thistle showed that *C. rubiginosa* in combination with tall fescue grass was successful in controlling the weed. Two *Urophora* species, *U. affinis* and *U. quadrifasciata*, are now well established in Virginia on spotted knapweed. *U. affinis* has dispersed more slowly than *U. quadrifasciata*. The former was deliberately released in Virginia for spotted knapweed control, but the latter moved into Virginia from releases in adjacent states. These two flies have reduced knapweed densities at sites where they are present in large numbers. Interspecific competition studies between the two *Urophora* species are under investigation. Inoculative releases of three exotic agents, *Hylobius transversovittatus*, *Galerucella californiensis*, and *Galerucella pusilla*, for the control of purple loosestrife in Virginia have been successful. *H. transversovittatus* is established in Goshen, in west central Virginia. Its establishment in Coeburn in southwestern Virginia was interrupted when town workers dredged the site. The two *Galerucella* species have become well established at several sites in southwest Virginia. Significant increases in the population of the two leaf beetle species have significantly defoliated and subsequently reduced flowering of purple loosestrife at these sites.

Keywords

Cassida rubiginosa, *Galerucella californiensis*, *Galerucella pusilla*, Chrysomelidae, *Rhinocyllus conicus*, *Trichosirocalus horridus*, *Hylobius transversovittatus*, Curculionidae, *Puccinia carduorum*, Pucciniaceae, *Urophora affinis*, *Urophora quadrifasciata*, Tephritidae, *Metzneria paucipunctella*, Gelechiidae, musk thistle, *Carduus thoermeri*, (= *nutans*), plumeless thistle, *Carduus acanthoides*, Canada thistle, *Cirsium arvense*, spotted knapweed, *Centaurea maculosa*, purple loosestrife, *Lythrum salicaria*

Introduction

Five invasive weeds have been the focus of classical biological control in Virginia in recent years. These are: musk thistle, *Carduus thoermeri* Weinmann = *C. nutans* L.;

plumeless thistle, *Carduus acanthoides* L.; Canada thistle, *Cirsium arvense* (L.) Scop.; spotted knapweed, *Centaurea maculosa* Lam., all in the family Asteraceae (Compositae); and purple loosestrife, *Lythrum salicaria* L. (Lythraceae). All are introduced weeds from Europe or Asia. Musk thistle and plumeless thistle are biennials. Canada thistle, spotted knapweed, and purple loosestrife are perennials. The three thistles and spotted knapweed are mainly pasture weeds, weeds in wasteland, and along the median strips of highways and side embankments throughout Virginia, except for the southeastern portion of the state. Purple loosestrife invades and degrades wetlands over much of the temperate United States and Canada and is found in southwestern and eastern Virginia. All the weeds have reproduced and multiplied successfully in the absence of their natural enemies. Thus, classical biological control was initiated by importing exotic natural enemies of the weeds from their native distribution into Virginia. This report summarizes the research and progress in the biological control of these weeds during the past decade.

Experimental Procedures

Working in conjunction with the US Department of Agriculture ARS, Agriculture Canada, and the International Institute of Biological control, several biological control agents were imported (Table 1). Releases of the exotic biological control agents at selected release sites were monitored for establishment and impact. Population dynamics, biology, and movement of the introduced biological control agents were investigated. Natural dispersal from the initial release sites was monitored and established populations of the biological control agents were collected and relocated to other areas infested with the target weeds. Annual surveys were conducted at each initial release site to determine the density of both biological control agents and target weeds. Transects were marked at each site for determination of the population dynamics of biological control agents as well as weeds.

Table 1.
Established biological control agents of invasive weeds in Virginia

<u>Target weed</u>	<u>Biological control agent</u>
<i>Carduus thoermeri</i> Weinmann = <i>C. nutans</i> L. [Asteraceae=Compositae] {musk thistle, nodding thistle}	<i>Cassida rubiginosa</i> Müller [Coleoptera: Chrysomelidae] <i>Rhinocyllus conicus</i> Froelich [Coleoptera: Curculionidae] <i>Trichosirocalus horridus</i> (Panzer) [Coleoptera: Curculionidae] <i>Puccinia carduorum</i> Jacky [Uredinales: Pucciniaceae]
<i>Carduus acanthoides</i> L. [Asteraceae=compositae] {Plumeless thistle}	<i>Trichosirocalus horridus</i> (Panzer) [Coleoptera: Curculionidae]
<i>Cirsium arvense</i> (L.) Scop. [Asteraceae=Compositae] {Canada thistle}	<i>Cassida rubiginosa</i> Müller [Coleoptera: Chrysomelidae]
<i>Centaurea maculosa</i> Lam. [Asteraceae=Compositae] {Spotted knapweed}	<i>Urophora affinis</i> Fraunfeld [Diptera: Tephritidae] <i>Urophora quadrifasciata</i> (Meigen) [Diptera: Tephritidae] <i>Metzneria paucipunctella</i> Zeller [Lepidoptera: Gelechiidae]
<i>Lythrum salicaria</i> L. [Lythraceae] {Purple loosestrife}	<i>Hylobius transversovittatus</i> Goeze [Coleoptera: Curculionidae] <i>Galerucella californiensis</i> (L.) <i>Galerucella pusilla</i> (Duft.) [Coleoptera: Chrysomelidae]

Results and Discussion

Musk thistle. Four biological control agents have become well established for musk thistle control. Two are weevils, *Rhinocyllus conicus* Froelich and *Trichosiromus horridus* (Panzer) (Coleoptera: Curculionidae), the third is a leaf beetle, *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae), and the fourth is a fungus, *Puccinia carduorum* Jacky (Uredinales: Pucciniaceae). Except for *C. rubiginosa*, which accidentally entered eastern North America, all were deliberately released for musk thistle control.

R. conicus is a thistle head weevil imported from France that was first released in Virginia in 1969 (Surles *et al.* 1974). Eggs are laid on the bracts. Developing larvae feed on both the receptacle and the young achenes, significantly reducing seed production. *T. horridus* was imported from Italy and first released in 1974 (Kok and Mays 1989). Eggs are laid in the mid-rib of the underside of the leaves, usually in clusters of two or three. Larvae feed on crown tissue of rosettes

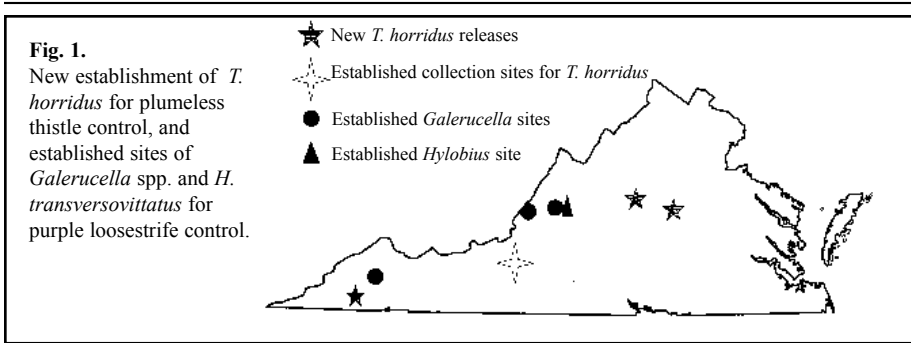
With field establishment of the two weevils, *R. conicus* and *T. horridus*, musk thistle reductions exceeding 90% of the initial thistle density are common in Virginia (Kok 1986, Kok 1998). *T. horridus* overwinters in the egg, larva, and adult stages (Kok and Mays 1989) and this allows the insect to survive severe winters better than *R. conicus*, which overwinters only in the adult stage (Kok 1998). Musk thistle reduction occurred in about five to six years, about half the time as that of plumeless thistle (Kok and Mays 1991).

The other two established biological control agents were *C. rubiginosa*, and *P. carduorum*. *C. rubiginosa*, a leaf beetle, was accidentally introduced into eastern North America (Barber 1916) and spread south to Virginia (Cartwright and Kok 1990). Both adults and larvae feed on the leaves. It survives better on Canada thistle than musk thistle although no preference in oviposition was observed between the two thistle species (Spring and Kok 1997). Impact of feeding by this beetle is greatest on the young rosettes, as defoliation of thistles after the plant has bolted produced little effect (Cartwright and Kok 1990).

The rust fungus was imported from Turkey and primarily attacks musk thistle. It causes rapid senescence in the plant and adds to the stress on musk thistle by the three beetles, and is also transmitted by the herbivores (Kok and Abad 1994, Kok *et al.* 1996, Kok 1998). The rust fungus was first introduced into western Virginia in 1987 (Baudoin *et al.* 1990). It successfully overwintered under Virginia conditions (Baudoin *et al.* 1993), spread rapidly, and was located up to 500 km from the release site by 1992 (Baudoin and Kok 1994). By 1994, it was in north central Missouri (Baudoin and Bruckart 1996).

The four biological control agents complement each other and increased the stress on musk thistle, resulting in significantly reduced seed production. Based on the results to date, we feel that no further biological control agents need be introduced into Virginia or the USA for musk thistle control.

Plumeless thistle. Of the four biological control agents mentioned above, only *T. horridus* shows effective control of the plumeless thistle, but it takes twice as long to control plumeless thistle than that of musk thistle (Kok 1986, Kok and Mays 1991). *R. conicus* provides only partial control as it infests the primary flower buds. The lateral buds are out of synchrony with the oviposition of *R. conicus* (Kok 1998) because they develop after the weevil has stopped laying eggs. *C. rubiginosa* feeds lightly on the leaves and the rust fungus does not infect it. Thus, more biological control agents would be needed to expedite the control of plumeless thistle. Search for a host specific pathogen that would



complement the activity of *T. horridus* would be a good addition for the control of this weed. In the meantime, we have established two field nurseries for the propagation of *T. horridus* for relocation to areas with heavy infestations of plumeless thistle. Establishment of the field nurseries was based on the results of a study by Stoyer and Kok (1986). Recent releases have concentrated on counties (Fig. 1) with plumeless thistle that have had few releases of the weevil in previous years. In sites that initially have a mixture of both musk and plumeless thistle, the musk thistle is controlled after a few years, although the plumeless thistle persists.

Canada thistle — *C. rubiginosa* is the most conspicuous defoliator of Canada thistle in southwestern Virginia since its establishment. Thistle growth was inhibited when defoliation exceeds 50% of the leaf tissue (Cartwright and Kok 1990). The impact is greater on rosettes than on bolted plants. With 20 beetles per rosette, less than one-third of the plants survived (Ang et al. 1995). Despite the abundance of larval and pupal parasitoids, this leaf beetle has become widespread in southwest Virginia (Ang and Kok 1995). Field studies indicated that this beetle shows no preference for oviposition on either musk or Canada thistle. Development of egg to adult stage was completed in 27.4 days on Canada thistle, 1.1 day shorter than that on musk thistle (Spring and Kok 1997). The major difference was greater mortality of the immature stage when feeding on musk thistle compared with Canada thistle. About one in five eggs completed development to the adult stage when feeding on musk thistle; two in five completed development on Canada thistle. *C. rubiginosa* in combination with tall fescue effectively suppressed Canada thistle. Studies on the competitive growth of Canada thistle, tall fescue, and *C. rubiginosa* indicate that the beetle and induced plant competition can be effective as part of a comprehensive approach for area-wide Canada thistle control (Ang et al. 1994). Both *C. rubiginosa* and tall fescue significantly reduced biomass and survival of Canada thistle (Ang et al. 1995). Introduction of plant competitors early in the establishment of thistles gives the best control (Ang et al. 1993). Thus, long-term control of Canada thistle is achievable with *C. rubiginosa* and plant competition.

Spotted Knapweed. Two European gall-producing insects in seedheads, *Urophora affinis* Frfld. (Diptera: Tephritidae) and *Metzneria paucipunctella* (Zeller) (Lepidoptera: Gelechiidae) were introduced into Virginia in 1986 (Kok 1989). Larvae of the seed-head fly, *U. affinis*, and the moth, *M. paucipunctella*, feed on seeds and receptacle. Adults of *U.*

affinis (n = 2625) and *M. paucipunctella* (n = 450) were released at two sites in Montgomery County, and their populations were monitored yearly by dissecting spotted knapweed flower heads. Beginning in 1992, knapweed samples collected at various distances from the release sites were checked for dispersal. *U. affinis* is well established and is spreading slowly. The number of larvae per flower head and the seed numbers are inversely related. Plants with the greatest number of larvae per spotted knapweed head had the lowest number of seeds. Knapweed density declined at the sites with high rates of infestation (Mays and Kok 1996). At several of the release sites, a second species, *Urophora quadrifasciata* (Meigen), was recovered in 1996. *U. quadrifasciata* was not approved for release in Virginia, and has spread naturally from releases in neighboring states. It was reported to be spreading rapidly in the northeastern states (Hoebeker 1993), and because its dispersal rate is much faster than that of *U. affinis* (Harris and Myers 1984), it is likely to have moved south into Virginia. We are currently monitoring the movement of both species. Establishment of *M. paucipunctella* is tentative. Its population has been low despite being recovered for several years between 1995-1997. It was found in low numbers among samples with *U. affinis*. However, since the influx of *U. quadrifasciata*, it has not been recovered and was not found among the samples in 1999.

Purple Loosestrife. Three exotic beetles, *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), *Galerucella californiensis* (L.) and *G. pusilla* (Duft.) (Coleoptera: Chrysomelidae), were introduced into Virginia for biological control of purple loosestrife, *Lythrum salicaria* L. (Lythraceae). Quarantine studies on host specificity were completed in 1992 (Kok *et al.* 1992a, b) and initial releases of each species were approved and made in Coeburn, Virginia in 1992 (McAvoy *et al.* 1997, McAvoy and Kok 1999).

The two *Galerucella* beetles are leaf feeders, and have become well established in the original release site in Coeburn, and in two other sites in southwestern Virginia (Fig. 1). Eggs are laid in batches on the basal part of the stem, shoot axils, or lower side of leaves. Larvae feed at the top of the plant moving downward. The leaf beetles have shown steady increases for at least three consecutive years (McAvoy *et al.* 1997). Dispersal, as measured by the area covered by the beetles, has been slow. Overwintering adults and eggs are found in early May, followed by larvae in late May through mid June. The F₁ adults emerge and feed briefly in late June and early July before going into diapause. Our recent data (June 1999, unpublished) indicated that as much as 70% of the purple loosestrife at one of the field sites has been completely defoliated by the leaf beetles.

H. transversovittatus is a root feeder. Eggs are laid either into the lower part of the main shoot or onto the root below ground level. Although the establishment of the weevil at the first release site in Coeburn was interrupted by dredging along the river, it has become established in the second release site (Goshen) where we have recovered larvae for two consecutive seasons in increasing numbers. Adult females reared at constant temperatures in the laboratory showed that longevity is inversely related to temperature (McAvoy and Kok 1999). Females lived for over three years at the lower temperatures, and survived six times longer at 12.5°C than at 30°C. Developmental data indicate that 25°C is the optimum temperature for oviposition and egg development. These temperature conditions are common in the early spring through fall in Virginia and would suggest that the weevil should have no difficulty getting established. This bodes well for the biological control of purple loosestrife in Virginia.

References

- Ang, B.N., and L.T. Kok. 1995.** Seasonal mortality of *Cassida rubiginosa* (Coleoptera: Chrysomelidae) due to incomplete development and parasitism in southwestern Virginia. *J. Entomol. Sci.* 30: 9-17.
- Ang, B.N., L.T. Kok, G.I. Holtzman, and D.D. Wolf. 1993.** Response of various ages of Canada thistle (*Cirsium arvense*) to *Cassida rubiginosa* (Coleoptera: Chrysomelidae) and density of tall fescue and crownvetch. *Entomol. (Trends Agric. Sci.)* 1: 45-53.
- Ang, B.N., L.T. Kok, G.I. Holtzman, and D.D. Wolf. 1994.** Competitive growth of Canada thistle, tall fescue, and crownvetch in the presence of a thistle defoliator, *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae). *Biological Control* 4: 277-284.
- Ang, B.N., L.T. Kok, G.I. Holtzman, and D.D. Wolf. 1995.** Canada thistle [*Cirsium arvense* (L.) Scop.] response to density of *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae) and plant competition. *Biological Control* 5: 31-38.
- Barber, H.S. 1916.** A review of North American tortoise beetles. *Proc. Entomol. Soc. Wash.* 18: 113-127.
- Baudoin, A.B.A.M., and W.L. Bruckart. 1996.** Population dynamics and spread of *Puccinia carduorum* in the eastern United States. *Plant. Dis.* 80: 1193-1196.
- Baudoin, A.B.A.M., and L. T. Kok. 1994.** Rust disease introduced for biological control of musk thistle becomes widespread. In "Virginia Integrated Pest Management", C. Laub and F. W. Ravlin [eds.], pp. 29-30. Virginia Polytechnic Institute and State University, Blacksburg, VA.
- Baudoin, A.B.A.M., R.G. Abad, L.T. Kok, and W.L. Bruckart. 1990.** Development of *Puccinia carduorum* and its impact on musk thistle in Virginia. (Abstr.) *Phytopathology* 80: 1011.
- Baudoin, A.B.A.M., R.G. Abad, L.T. Kok, and W.L. Bruckart. 1993.** Field evaluation of *Puccinia carduorum* for biological control of musk thistle. *Biological Control* 3: 53-60.
- Cartwright, B., and L.T. Kok. 1990.** Feeding by *Cassida rubiginosa* (Coleoptera: Chrysomelidae) and the effects of defoliation on growth of musk thistles. *J. Entomol. Sci.* 25: 538-547.
- Harris, P., and J.H. Myers. 1984.** *Centaurea diffusa* Lam. and *C. maculosa* Lam., diffuse and spotted knapweed (Compositae). In "Biological Control Programmes Against Insects and Weeds in Canada 1969-1980", J. S. Kelleher and M. A. Hulme [eds.], pp. 127-137. CAB, Slough, England.
- Hoebeke, E.R. 1993.** Establishment of *Urophora quadrifasciata* (Diptera: Tephritidae) and *Chrysolina quadrigemina* (Coleoptera: Chrysomelidae) in portions of the Eastern United States. *Entomol. News* 104: 143-152.
- Kok, L.T. 1986.** Impact of *Trichosirocalus horridus* (Coleoptera: Curculionidae) on *Carduus* thistles in pastures. *Crop Prot.* 5: 214-217.
- Kok, L.T. 1989.** Biological control of weeds in Virginia from 1969-86. *Proc. VII. Int. Symp. Biol. Contr. Weeds*, 6-11 March 1988, Rome, Italy. E. S.Delfosse, [ed.], *Ist. Sper. Patol. Veg. (MAF)*, pp. 623-9.
- Kok, L.T. 1998.** Biological control of musk and plumeless thistles. *Recent Res. Devel. In Entomol.* 2: 33-45.
- Kok, L.T., and R.G. Abad. 1994.** Transmission of *Puccinia carduorum* by the musk thistle herbivores, *Cassida rubiginosa* (Coleoptera: Chrysomelidae), *Trichosirocalus horridus* and *Rhinocyllus conicus* (Coleoptera: Curculionidae). *J. Entomol. Sci.* 29: 186-191.
- Kok, L.T., and W.T. Mays. 1989.** Comparison of the seasonal occurrence of *Trichosirocalus horridus* (Panzer) (Coleoptera: Curculionidae) in Virginia between 1981-83 and 1979. *J. Entomol. Sci.* 24: 465-471.
- Kok, L.T., and W.T. Mays. 1991.** Successful biological control of plumeless thistle, *Carduus acanthoides* L. [Campanulatae: Asteraceae (= Compositae)], by *Trichosirocalus horridus* (Panzer) (Coleoptera: Curculionidae) in Virginia. *Biological Control* 1: 197-

202.

- Kok, L.T., R.G. Abad, and A.B.A.M. 1996.** Effects of *Puccinia carduorum* on musk thistle herbivores. *Biological Control* 6: 123-129.
- Kok, L.T., T.J. McAvoy, R.A. Malecki, S.D. Hight, J.J. Drea, and J.R. Coulson. 1992a.** Host specificity tests of *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a potential biological control agent of purple loosestrife, *Lythrum salicaria* L. (Lythraceae). *Biological Control* 2: 1-8.
- Kok, L.T., T.J. McAvoy, R.A. Malecki, S.D. Hight, J.J. Drea, and J.R. Coulson. 1992b.** Host specificity tests of *Galerucella californiensis* (L.) and *G. pusilla* (Duft.) (Coleoptera: Chrysomelidae), potential biological control agents of purple loosestrife, *Lythrum salicaria* L. (Lythraceae). *Biological Control* 2: 282-290.
- Mays, W.T., and L.T. Kok. 1996.** Establishment and dispersal of *Urophora affinis* (Diptera: Tephritidae) and *Metzneria paucipunctella* (Lepidoptera: Gelechiidae) in southwestern Virginia. *Biological Control* 6: 299-305.
- McAvoy, T.J., L.T. Kok, and W.T. Mays. 1997.** Phenology of an established population of *Galerucella californiensis* (L.) and *G. pusilla* (Duft.) (Coleoptera: Chrysomelidae) on purple loosestrife, *Lythrum salicaria* L. (Lythraceae), in southwest Virginia. *Biological Control* 9: 106-111.
- McAvoy, T.J., and L.T. Kok. 1999.** Effects of temperature on eggs, fecundity, and adult longevity of *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a biological control agent of purple loosestrife. *Biological Control* 15: 162-167.
- Spring, A., and L.T. Kok. 1997.** Field studies on the reproductive capacity and development of *Cassida rubiginosa* (Coleoptera: Chrysomelidae) on musk and Canada thistles. *Environ. Entomol.* 26: 876-881.
- Stoyer, T.L., and L.T. Kok. 1986.** Field nurseries for propagating *Trichosirocalus horridus* (Coleoptera: Curculionidae), a biological control agent for *Carduus* thistles. *J. Econ. Entomol.* 79: 873-876.
- Surles, W.W., L.T. Kok, and R.L. Pienkowski. 1974.** *Rhinocyllus conicus* establishment for biocontrol of thistles in Virginia. *Weed Sci.* 22: 1-3.