Field Evaluation of a Gall Mite for Biological Control of False Cleavers

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1 Abstract

The European gall mite *Cecidophyes rouhollahi* Craemer (Acari: Eriophyidae) was imported into Alberta from southern France, and a breeding colony was set up for evaluation of its potential as a biological control agent for false cleavers, *Galium spurium* L. (Rubiaceae), an important weed of canola and other annual crops in Alberta. Field plots were set up in 2003 and 2004 to evaluate the mite's rate of development, increase and dispersal under field conditions in Alberta, to evaluate its impact on the growth and seed production of false cleavers when applied at different growth stages of the weed, and to assess its ability to survive over winter in the field in Alberta under various cultural conditions. The mite successfully colonized false cleavers plants growing in plots both with and without canola. In 2004, false cleavers plants inoculated with mites at the 2 leaf-whorl stage experienced a 31.2% reduction in aboveground biomass and a 33% reduction in seed production compared with uninfested plants. Early inoculation of mites (at the 2 leaf-whorl stage) resulted in heavier galling and greater reductions in biomass than inoculation at the 6 leaf-whorl stage. Although some apparent wind-borne dispersal of mites was observed in the field, mite populations usually remained very localized and patchy, even within plots. Individual false cleavers stems were often heavily galled while adjacent stems remained uncolonized. Overwinter survival of mites in the field has not yet been confirmed. Successful implementation of this mite as a biological control agent for false cleavers will depend on the development of application methods to provide more uniform coverage, resulting in more complete and extensive galling.

2 Background and Objectives

False cleavers is a major and increasing weed of canola and other crops in Alberta. Surveys indicate that during the 1990s, in each of the Prairie Provinces, it increased its abundance ranking more rapidly than any other cropland weed (Thomas et al. 1998a; 1998b; 1998c). In Alberta, for example, it occurred in less than 1% of cereal and oilseed fields surveyed in 1973-77 (Thomas and Wise 1985), and 18% of fields surveyed in 2001 (Leeson et al. 2002). Heavy infestations cause yield losses by competing with canola. False cleavers seed cannot be separated easily from canola seed, leading to contamination and downgrading of the crop. False cleavers contamination of canola seed also results in new infestations. *Galium spurium* is listed as noxious under the Alberta Weed Control Act and the closely related *Galium aparine* is listed as noxious in all the Prairie Provinces. In 1996 the first case of herbicide resistance in *G. spurium* was discovered in Alberta, involving multiple resistance to chemicals in different groups (Hall et al. 1998). This population is highly resistant to the ALS-inhibiting herbicides triasulfuron, thifensulfuron/ tribenuron, and sulfometuron, and moderately resistant to the ALS inhibitor imazethapyr; it is also cross-resistant to the auxin-type herbicide quinclorac. Imazethapyr is one of the products for which herbicide-tolerant canola has been developed, and is also registered for control of false cleavers in field peas. Increased herbicide use in herbicide-tolerant crops is likely to lead to an increased incidence of herbicide resistance in false cleavers. The need for alternative management tools for false cleavers is therefore likely to become more urgent.

A previously undescribed gall mite, *Cecidophyes rouhollahi*, has been discovered in Europe that causes severe damage to cleavers (*Galium aparine* L.) and false cleavers (Craemer et al. 1999). In previous work funded by CARP and AARI, the biology and host specificity of the gall mite were studied by Dr. R. Sobhian at the USDA-ARS European Biological Control Laboratory in

Montpellier, France. Results showed that the mite is highly host-specific to a few closely related annual species of *Galium*, which are all weeds in North America, and does not attack any of the native North American perennial *Galium* species. The mite breeds rapidly and causes severe stunting, distortion, and yellowing of false cleavers. In greenhouse studies in France, attacked plants suffered 40% mortality, with surviving plants showing a 60% biomass reduction and a complete suppression of seed production (Sobhian et al. 2004).

A petition was submitted in March 2001 to CFIA and the USDA-APHIS Technical Advisory Group requesting approval of field release of *C. rouhollahi* in Canada (McClay et al. 2001). Final approval for field release of *C. rouhollahi* in Canada was received in a letter dated June 6 2002 from the Canadian Food Inspection Agency (see Appendix).

The objectives of this study were:

- To establish a culture of the gall mite *Cecidophyes rouhollahi* for field studies in Alberta

- To estimate the rates of development, increase and dispersal of the mite on false cleavers under field conditions in Alberta

- To evaluate the impact of the mite on growth of false cleavers when applied at different growth stages of the weed.

- To assess the mite's ability to survive over winter in the field in Alberta under various cultural conditions.

Additional laboratory studies were conducted to provide information on the mites' rate of reproduction and development under favourable conditions in the laboratory, and on their survival under low temperatures.

As there was an old report of possible plant virus-like particles associated with galling of *G. aparine* by this or a similar mite (Moha 1972), and as some plant viruses are known to be transmitted by eriophyid mites (e.g. Harvey and Seifers 1991; Gispert et al. 1998; Kulkarni et al. 2002), it was also necessary to conduct virus testing on the imported mite population to ensure that it was free of detectable viruses before field release. Such tests were conducted on the population originally screened and approved for introduction, and no virus contamination was detected (Sobhian et al. 2004). However, as the mites introduced in the present project were a separate colony based on fresh collections from the field, it was necessary to repeat this testing.

This project was initiated while the principal investigator was employed with the Alberta Research Council, Vegreville. On April 15, 2004, funding was transferred to McClay Ecoscience from the Alberta Research Council. Plot space and greenhouse facilities were used at ARC Vegreville.

3 Experimental Methods

3.1 Importation

Mites were collected in 2002 and 2003 by Dr. R. Sobhian (USDA) from *Galium aparine* at the original field site at Carnon (43°32' N, 3°59'E), near Montpellier, France, from which *C. rouhollahi* was described. Galled stems of *G. aparine* were wrapped in damp cotton wadding and shipped by air freight from Montpellier. In 2002, two shipments were received in Calgary and transferred to the quarantine facility of the Agriculture and Agri-Food Canada Research Station in Lethbridge for opening and transfer of mites. In 2003 the Lethbridge quarantine facility had been closed, and approval was obtained from the Canadian Food Inspection Agency to receive the shipment in Edmonton and process it at the Alberta Research Council laboratory in Vegreville, Alberta.

3.2 Colony establishment

Mites were transferred onto fresh young *Galium spurium* plants grown from seed in root trainers or 10-cm square pots by three methods:

1. "Leaf pieces": Galled leaves from the shipment were split open lengthwise to check for any contaminating organisms such as other insects or mites. These leaf pieces were then placed on the shoot tips of the fresh plants.
2. "Individual transfers": Individual mites were picked off the galled plants in the shipment under a dissecting microscope on the point of a 00 insect pin, and mites were transferred onto fresh plants.
3. "Stem pieces": Stem pieces including four leaf whorls were cut from the shipped material, inspected for contaminating organisms, and one stem piece per plant was laid onto the fresh plants.

From the first shipment in 2002, 4 plants were inoculated by method 3 and 3 plants by method 2. From the second shipment in 2002, five young *G. spurium* plants in root trainers were each inoculated by method 2 (30 – 50 mites transferred per plant) and two larger plants were inoculated by method 1 (6 leaf pieces per plant). In 2003, four plants were inoculated by method 1 (10 leaf pieces per plant), twelve by method 2 (100 mites transferred per plant), and four by method 3. All packing material and unused plant material from the shipment was autoclaved after the initial transfers had been made.

Mite samples from each shipment were preserved and sent to Dr. James Amrine, West Virginia University, for confirmation of identification. Voucher specimens were also sent to the Canadian Food Inspection Agency for deposit in the Canadian National Collection.

To minimize the possibility of carrying forward any undetected contaminating insect or mite species that might have been present in the leaf or stem pieces, only plants inoculated by individual transfers (method 2) were used as the source of mites for further colony maintenance. (The other methods were used initially as a backup in case transfer of individual mites did not result in successful colony establishment). When plants had been successfully colonized, the colony was maintained by periodically transferring infested stem pieces onto fresh potted *G. spurium* plants. The colony was maintained in cages in a growth chamber until the second set of virus tests (see below) had given negative results. The colony was then moved into a greenhouse.

3.3 Virus testing

After successful establishment of the laboratory colony in 2003, two sets of samples from *G. spurium* plants that had been infested with mites at ARC were sent to Agdia, Inc. (Elkhart, IN, USA) for virus testing, on April 7 and June 5, respectively. Each set of samples included fresh leaf tissue from mite-infested *G. spurium* plants from the laboratory colony and uninfested control plants from the same batch of seedlings. All samples were subjected to group PCR tests using primers that will detect known or unknown viruses in any of the following 13 groups: Begomovirus group, Bromovirus group (detects members of Alfamovirus, Bromovirus, Cucumovirus), Carlavirus group, Carmovirus group, Closteroviridae group, Dianthovirus group, Ilarvirus group, Luteovirus group (detects members of Luteovirus, Polerovirus, and unassigned viruses of Luteoviridae family), Nepovirus group (also detects Fabaviruses BBWV I & II), Potexvirus group, Potyvirus group (detects members of Potyvirus, Bymovirus, Tritimovirus, Ipomovirus), Tobamovirus group, and Tospovirus group (A. Harness, Agdia Inc., pers. comm. 2003). The second set of samples was tested only for those virus groups for which a positive test result had been found with the first series of samples.

3.4 Canola plots

The canola experiment set up in 2003 was a split-plot design with three tillage treatments (fall, spring, and none) as main plots, eight mite treatments as subplots, and four replicates. The subplot treatments were:

C	canola alone
G	false cleavers alone, no mites
GE	false cleavers alone, mites applied at 2 leaf-whorl stage
GL	false cleavers alone, mites applied at 6 leaf-whorl stage
CG	false cleavers in canola, no mites
CGE	false cleavers in canola, mites applied at 2 leaf-whorl stage
CGL	false cleavers in canola, mites applied at 6 leaf-whorl stage
W	late-seeded (winter annual) false cleavers, no canola, mites applied late summer

Plots were 2.25 × 3 m with 2.25 m spacing between subplots and 8 m borders between the main (tillage) plots. False cleavers was seeded where applicable at 100 seeds m^{-2} broadcast into a 1 m^2 central area on each plot on June 9 and conventional canola (Pioneer 46A65) was seeded on June 10. Mites were released by attaching 10 galled stem pieces from the greenhouse colony to false cleavers plants in the plot using short pieces of twist tie (Figure 1). The early mite releases (2 leaf-whorl stage of false cleavers) were made on July 2 and the late releases (6 leaf-whorl stage) on July 18.

The W treatment was added to investigate the possibility that the mites may require green (winter annual) false cleavers plants to survive over winter in the field. These plots were seeded with false cleavers on July 14 and inoculated with mites from the greenhouse colony on September 19. Because of limited availability of mites, only the main plots allocated to the no-tillage treatment received mites.

False cleavers plants were rated for mite damage on an arbitrary scale of 0 to 4, where 0 is no visible galling, 1 is minimal, 2 is slight, 3 is moderate, and 4 is extensive. For canola seed yield

determination, one 1 m^{-2} quadrat was hand harvested from the centre of each plot, leaving stubble height at 10 cm, on September 10-12, 2003. At the same time, false cleavers plants were removed for biomass and seed determination.

Remaining canola was combined October 3. Canola plant residue was baled off the plots on October 7. Plots designated for fall tillage were cultivated to a 15 cm depth on October 10, 2003 and plots designated for spring tillage were tilled on May 14, 2004.

In 2004 the experiment was repeated. Design and layout were the same as in 2003 except that plots were 2.5 × 5 m with 4 m spacing between the subplots and 8 m borders between the main (tillage) plots. Plots were seeded with false cleavers on May 20, 2004. The seeding rate was increased to 500 seeds m^{-2} to obtain higher false cleavers densities. Canola was seeded May 28, 2004. Early mite treatments were applied on June 22 and the late treatments were applied on July 6. Twelve infested stem pieces were applied per plot. Gall damage levels were monitored on July 20, July 28, August 4, and August 12. On September 2 the W plots were mowed and tilled to provide a seedbed. One plot in treatment GE was also accidentally mowed, and data from this plot were treated as missing. Cleavers and canola were harvested from September 17 to 23, and processed as in 2003.

Tillage treatments were not applied as planned in 2004. This was because of the very low levels of green false cleavers present in the plots after harvest. It was decided to treat all plots as no-tillage in order to maximize the possibility of overwinter survival of mites.

As it was observed in spring 2004 that winter annual false cleavers appeared to survive best in the cotyledon or early 1 leaf-whorl stage, W plots were seeded later than in 2003. False cleavers was seeded in these plots on September 10, 2004, at 5,000 seeds m^{-2} in a central 1 m^2 of the plot, to obtain a dense carpet of overwintering seedlings. Seedlings were inoculated on October 13, 2004, with mites from a culture that had been maintained indoors under lights. Approximately 3.8 g per plot (fresh weight) of heavily galled false cleavers stems containing abundant mites were finely chopped with scissors and scattered over the seedlings.

An additional overwintering test was set up on October 23, 2004, using 10-cm square plastic pots of mite-infested false cleavers that had been kept outdoors since August. A tray of 15 pots was dug into a garden with the soil in the pots flush with the soil surface, and covered with snow to a depth of approximately 15 cm (level with the surrounding natural snow cover).

3.5 Dispersal plots

The dispersal experiment was seeded on June 6, 2003 with false cleavers in two continuous 8-m strips forming a cross pattern and 52 isolated mini-plots, each 20 cm square, at varying distances and in all directions out from the centre of the cross pattern (Figure 2). The concentric circles of isolated mini-plots had radii of 0.8, 1.4, 2.4, and 3.6 m. An infested plant was transplanted into the centre of the cross pattern on July 18, 2003.

The dispersal experiment was repeated in 2004. False cleavers was seeded on June 3, 2004, more densely than in 2003, to obtain more continuous strips in the central cross pattern. Mites were applied at the centre of the experiment on July 6, 2004.

3.6 Field release

Although open field releases were not an original part of the proposal, one field release of *C. rouhollahi* was made on July 4, 2003, in dense false cleavers along the edge of a canola field near Mundare, Alberta. Four separate releases of galled stems were made at locations within an area of about 0.6 ha. At each site, stem pieces infested with mites were laid onto the false cleavers plants over an area of about 1 m^2.

A release was also made near Spirit River, Alberta, on June 30, 2004. Galled stems were placed on false cleavers plants in a field of creeping red fescue, in each of four 30 × 30 cm quadrats.

3.7 Development and reproduction at 25°C

The reproductive rate experiment was run from January 21 to February 5, 2004. Single adult mites from the greenhouse colony were placed on each of 140 false cleavers seedlings at the 2 leaf-whorl stage, in 4-cm square root trainers. Seedlings were placed in a growth chamber at constant 25°C, with 16:8 photoperiod. Ten seedlings were removed each day for 14 days and the number of eggs, immature and adult mites on each were recorded.

3.8 Low temperature survival

The low temperature survival experiment was run from February 23 to March 18, 2004. Eighty mite-infested stem tips from the greenhouse colony were placed individually in 2 ml snap-cap plastic centrifuge tubes. Ten each of these tubes were placed at each of 8 different storage temperatures (4°C, 0°C, -5°C, -10°C, -15°C, -20°C, -25°C, -80°C), and one tip removed from each temperature at 10 intervals from 1 to 24 days after start. Mite survival was determined by placing each tip on an uninfested false cleavers seedling in a 10-cm pot, and monitoring these seedlings for 7 days. Gall development indicated that at least one viable mite or egg had survived that storage treatment.

4 Results

4.1 Importation and colony establishment

4.1.1 Importation in 2002

Dr. R. Sobhian (USDA, Montpellier) found no mites up to April 2, 2002, at the field site in Carnon, France, where the mite was originally collected, although the host plant *Galium aparine* was common. At this site the mite had been abundant in previous years, usually beginning to appear in February or March. A single mite-infested plant was found on April 3 at a site about 20 km from Carnon, and was used to set up a greenhouse colony on *Galium spurium* (false cleavers) in Montpellier. A shipment of about 50 infested stems from this colony was sent from Montpellier on May 14 and received in the quarantine facility of Agriculture and Agri-Food Canada at Lethbridge on May 16. The plant material was somewhat wilted on arrival and the level of mite damage was low. Seven small *G. spurium* plants were inoculated with mites, either by direct transfer of single mites or by attaching pieces of infested stems, and placed in growth chambers in the quarantine. All plants were inspected on June 7 and no sign of mite colonization

was found. Dr. J. Amrine confirmed the identity of mites from this shipment as *C. rouhollahi* (Figure 3).

On June 3, 2002, Dr. Sobhian found a few infested *G. aparine* plants at the Carnon site. A second shipment of field-collected stems was sent on June 4 and received in excellent condition on June 7. Five young *G. spurium* plants and two larger plants were inoculated as described above. Signs of mite damage were observed on June 14, and by June 19 all 5 plants inoculated with single mites were showing characteristic rolling of the apical leaves, suggesting mite colonies were developing. However, these leaves then dried up and turned brown and no further signs of damage were seen. All plants were thoroughly inspected on July 9 and no trace of mite infestation was found.

By July *G. aparine* plants in the field at Carnon were senescent and no further mite collections could be made. Further attempts to establish a colony of *C. rouhollahi* were postponed until 2003.

4.1.2 Importation and colony establishment, 2003

In 2003, mite damage was once again abundant on *Galium aparine* at the original Carnon collection site. A shipment of field-collected mites from this site was received on March 6, 2003. The plant material was received fresh and in good condition, with very abundant and vigorous gall mite populations, and was virtually free of any other mites or insects. Dr. Amrine again confirmed the identification of the gall mite as *C. rouhollahi*.

Mite establishment occurred rapidly on all inoculated plants. Leaf curling, indicating that mites were colonizing the plants, was seen within 3 days of mite transfer by all methods (Figure 4). Seven days after transfer, dissection of a shoot tip from a plant inoculated by method 2 showed a few adult mites and numerous eggs and immature stages. By 15 days after transfer, all plants were heavily galled. By 3 – 4 weeks after transfer, plants had many dry, brown leaf whorls, and stems were beginning to blacken at the nodes and collapse (Figure 5).

This gall mite colony has been maintained continuously to date by periodically reinoculating the mites onto fresh false cleavers plants. However, during hot weather in late June and early July 2003, mite performance and colony vigour in the greenhouse at ARC Vegreville seemed to decrease, with the result that only small numbers of lightly infested stems were available for inoculation into the plots on July 2 and 18, 2003.

4.2 Virus testing

Virus testing of the first set of samples (sent on April 7) showed that mite-infested plants from the growth chamber colony were positive for the carlavirus, luteovirus, and potyvirus groups (weakly positive in the case of potyvirus). Corresponding control samples were negative for all these groups. The test for the tobamovirus group was positive in both the mite-infested and control plants. All these virus groups consist overwhelmingly of viruses known to be vectored by aphids or, in a few cases, whiteflies, while no viruses in these groups are known to be mite-transmitted. As some aphids had been present on the *G. spurium* plants from the greenhouse used

for the initial transfers, it was suspected that these aphids might be the source of the positive virus tests.

The second set of samples was taken from plants which had been grown from seed in closed cages screened with fine mesh aphid-proof fabric, both before and after mite inoculation, and was sent to Agdia on June 5, 2003. All tests with both control and mite-infested samples from this second series were negative for all four virus groups (carlavirus, luteovirus, potyvirus, and tobamovirus). This is consistent with the hypothesis that aphid populations in the greenhouse were the source of the viruses detected in the earlier series of tests, and indicates that no evidence of viruses associated with the introduced mites could be found.

4.3 Canola plots

4.3.1 Results from 2003

The seeding rate of false cleavers used in 2003 was probably too low, as mean germination rate in the field was about 35%, not enough to produce a continuous cover of false cleavers. However enough plants were present to conduct the mite releases. On July 23-24, mite galling was seen on false cleavers in 20 of the 24 plots on which mites had been released early, and in all 24 plots on which mites were released late. However, most plots did not develop heavy galling by the end of the season; mean gall rating on September 10 was only 0.92 in plots without canola and 0.33 in plots with canola. No galling was seen on false cleavers in any of the control (no mites) plots.

Mean canola seed yield across all plots with canola was 154.6 g m^{-2} (1.546 tonne ha^{-1}). Presence or absence of false cleavers did not significantly affect canola yield (ANOVA for randomized complete block design with replication, $F=1.07$, $p= 0.308$) (Table 1). Biomass of false cleavers developing in the plots with canola was very low compared to the plots with no canola (Table 2), indicating that canola was able to effectively suppress growth and development of false cleavers. To avoid swamping of mite effects by the huge canola effect, mite effects on false cleavers were analyzed separately in plots with and without canola.

With false cleavers growing in canola, there were no significant differences in false cleavers biomass ($F=0.93$, $p=0.407$) or seed production ($F=0.40$, $p=0.676$) between the different mite treatments. However there was a trend for plots without mites to have the highest false cleavers seed production and biomass, and those where the mites had been applied early to have the lowest. Plants with mites applied late had significantly higher levels of visible gall damage in September than those where the mites had been applied early ($F=7.06$, $p=0.003$) (Table 3).

In plots with false cleavers growing without canola, there were no significant differences or clear trends in seed production or biomass between the different mite treatments. In these plots false cleavers grew into large, bushy plants, and there was still much green foliage on many of these plots in mid September. Levels of galling observed in September were again significantly higher in plots with late-applied mites compared to those with early-applied mites ($F=16.21$, $p<0.0001$) (Table 4).

All plots were checked thoroughly on May 19 and June 29, 2004 for possible overwinter mite survival. Overwintered or newly germinated false cleavers was found in all plots except the

canola-only plots from the 2003 experiment. No sign of damage to false cleavers indicating overwinter survival of mites was found in any of these plots.

4.3.2 Results from 2004

There was good rainfall immediately after seeding the canola in the 2004 plots; conditions became dry by late June, but from July onwards moisture levels were generally high. False cleavers and canola germinated more or less simultaneously. Germination of false cleavers was good , with a density of 100 – 130 seedlings m^{-2} in most plots. Gall development was seen by July 20 in all plots that had received mite treatments, and in none of the control plots.

False cleavers biomass and seed production were greatly reduced in plots with canola compared to those without (reductions of 83.7% and 84.0% respectively), indicating that canola was able to suppress false cleavers growth to a large extent. The proportion of false cleavers biomass allocated to seed production was, however, not affected significantly by the presence of canola (Table 5).

Mean canola seed yield over the experiment was 227.8 g m^{-2} (2.278 tonnes ha^{-1}). Canola yield was not affected significantly by false cleavers; yield was virtually identical between plots with and without false cleavers, nor was there a significant effect of false cleavers biomass on canola seed yield among plots in which both species occurred.

Levels of gall development were significantly affected by both time of inoculation and presence of canola (Figure 6). This figure shows mean gall ratings for August 4, which was the date on which these ratings were highest. Early application of mites (at about the 2 leaf-whorl stage, on June 22) led to significantly higher levels of galling than late application (at about the 6 leaf-whorl stage, on July 6). For the early inoculation date, plots without canola had significantly higher levels of galling than those with canola; a similar trend was seen for the late inoculation date but this was not significant. With the exception of one false cleavers-only plot that was rated at 2 on August 12, no mite galling was seen on any plot that had not been inoculated with mites. Thus it appears that mites remained largely confined to the plots on which they had been inoculated.

There was a significant overall effect of mite treatments on false cleavers total aboveground biomass (Table 6). In plots without canola, false cleavers biomass was reduced by 29.6% in the early mite inoculation treatment, compared with uninoculated control plots (Figure 7). Plots with the late inoculation treatment had an intermediate reduction in biomass. A similar trend was seen in plots with canola, where false cleavers biomass was reduced by 31.2% in the early mite inoculation treatment, compared with uninoculated control plots, and the biomass in the late inoculated plots was intermediate. Although there was no significant difference among mite inoculation dates within the plots with canola, the lack of a significant interaction between canola and mite treatments (Table 6), and the very similar percent reduction in false cleavers biomass between plots with and without canola, suggest that the mites had a similar impact on false cleavers biomass in the canola plots as they had in the plots without canola.

The effects of mite and canola treatments on false cleavers seed production were also significant, and very similar to those on biomass (Table 7). In plots without canola, seed production was

reduced by 33.0% in plots that received the early mite inoculation compared with uninoculated control plots. The later inoculated plots had an intermediate level of reduction (Figure 8). A similar trend was seen in the plots with canola, where the early inoculated plots also showed a reduction of 33.0% in seed production compared with the uninoculated controls.

Mite treatments had no significant effects on the percentage of false cleavers biomass allocated to seed production; this ratio was very similar in plots with and without canola (Figure 9).

4.4 Dispersal plots

Because of the low seeding rate used in the 2003 dispersal experiment, the central "cross" pattern did not develop into continuous strips of cleavers, but instead had a number of gaps (Figure 2). Galled leaves were found on a number of the isolated "mini-plots", up to 3.6 m out from the central release point (Figure 10), indicating that mites were able to disperse over bare ground across gaps of up to 1.2 m between false cleavers plants. The first of these dispersed colonies was found 12 days after the inoculation, on July 30, 2003. This dispersal was probably wind-borne. Dispersal was not strongly directional but there appeared to be a slight tendency for greater dispersal to the east, probably reflecting prevailing winds. All gall damage found outside the central release point was very minor in extent.

In 2004, as a result of higher seeding rates, the central strips were more or less continuous. However dispersal of mites was less than that observed in 2003 (Figure 11). Again there appeared to be a slight tendency for greater dispersal to the south and east.

4.5 Field releases

The release site at Mundare was checked on August 1, 2003. No sign of galling was found on false cleavers plants around any of the release stakes.

The Spirit River release site was checked on August 10, 2004. Plants were largely mature or senescent but some galling was found on plants in each of the four release quadrats. Some stems were well galled and appeared stunted compared to adjacent ungalled stems. Live mites were seen in the shoot tip of one galled stem.

4.6 Development at 25°C

Eggs were first found 2 days after placing adult mites on the plants, newly hatched larvae after 5 days and new adults after 9 days. This suggests that the mite's life cycle from egg to adult can be completed in about 7 days at 25°C. By 14 days after the start of the experiment, seedlings contained a mean of 648 mites in all stages (range 286 – 1165), all being the descendents of a single mated female. Daily egg production is difficult to estimate directly from these data but is probably in the range of 2 – 4 eggs per female per day. Thus the mite population can increase extremely rapidly under favourable conditions (Figure 12).

4.7 Low temperature tolerance

Results are shown in Table 8. Mites survived up to 24 days at temperatures of –8°C and above and were subsequently able to initiate colony formation. Viability was only observed for up to 2

days when stored at –15°C or –20°C, and no survival was found at –80°C. (A single sample showed viability after 2 days at –80°C, but this was probably due to cross-contamination.)

5 Discussion

After initial delays due to the disappearance of the mite from the field site in France in 2002, and the need for virus testing of the population imported in 2003, a healthy laboratory colony of *C. rouhollahi* has been established and maintained to date. PCR testing of this colony for a wide range of virus groups gave no indication that the mites were associated with any plant virus.

The 2003 field experiments were delayed, due to the need for virus testing to be completed before mites could be released into the field, so that canola was seeded only on June 2. Population densities of false cleavers were low, particularly in the plots with canola. The numbers of mites available for release were also low, due to slow growth of the greenhouse colony during hot conditions in late June and early July. Thus the canola experiment in 2003 was not representative of typical production practices, or of the potential results of large mite releases. There was a trend for plots with mite releases to have lower biomass and seed production of false cleavers than those without mites, but this trend was not significant. Surprisingly, plots inoculated later with mites appeared to have better gall development than those inoculated earlier. It would be expected that early release would lead to better gall development, as the mites would have more time to multiply. It is possible that, because of the limited supply of mite-infested plants from the greenhouse colony, the plant material used for the late inoculation contained more mites than that used for the early inoculation, leading to the observed results.

In the 2004 field experiment, seeding times were more representative of typical production practices in the field, and good supplies of mite-infested false cleavers were available for both early and late inoculations. A higher seeding rate for false cleavers also led to more abundant populations of the weed both in plots with and without canola. In this experiment, early inoculation led to significantly higher levels of gall damage than late inoculation, as would be expected. Biomass and seed production of false cleavers were significantly reduced, by about 30% compared with plots without mites.

These results show that *C. rouhollahi* can have a significant impact on the growth and seed production of false cleavers under field conditions in Alberta. Its impact, however, was not as severe as that observed under greenhouse conditions by Sobhian et al. (2004), where biomass was reduced by 60% and seed production by 100%. This may be partly because field-grown false cleavers plants are more robust than potted plants in a greenhouse, or because mites multiplied more rapidly in the greenhouse experiment than under field conditions. However, observation of the plots suggested that one important explanation may be that mite colonization of the false cleavers plants was very uneven. Even in the most heavily galled plots, some plants or stems would show heavy damage while adjacent ones were free from visible mite damage. Under these circumstances, reductions in seed production or biomass of the attacked stems would tend to be compensated for by more vigorous growth of neighbouring attacked ones. This unevenness was probably a result of the inoculation technique used, in which small sprigs of infested false cleavers were attached to shoot tips of plants in the plot. This would have led to a concentration

of mites on the particular stems that happened to receive the initial inoculation, while other stems could have escaped damage.

These observations suggest that the impact of the mites could be enhanced by developing application methods that would distribute mites uniformly and thoroughly over all false cleavers plants in a stand. Scattering finely chopped infested plant tissue has been found to be an effective way of establishing the gall mite *Aceria malherbae*, an effective biological control agent for field bindweed (Michels et al. 1999; Britten et al. 2003).

Results of the dispersal experiment show that the mite can disperse short distances, up to 1.2 m, across open spaces between false cleavers plants, although these dispersal events did not result in the establishment of vigorous new colonies. In the canola plot experiments, no dispersal from plot to plot was observed with the exception of one possible event in the 2004 experiment. This suggests that the 2.25 m spacing between the plots used in 2003 and the 4 m spacing used in 2004 were generally sufficient to prevent mite spread. Dispersal will of course be dependent on the size of source populations; if heavy mite infestation can be achieved on large stands of false cleavers, dispersal over larger distances will probably be observed.

Only limited open field releases were made. No establishment was seen from the release made at Mundare in 2003. Galling was confirmed from the release made at Spirit River in 2004, but no dispersal outside the release quadrats was seen.

It has not yet been possible to confirm overwinter survival of the mites in the field in Alberta. The low temperature survival study suggested that the mites have limited ability to survive at temperatures below –10°C. This would imply that overwinter survival would be strongly dependent on snow cover to provide insulation. This study, however, was conducted with mites taken directly from a greenhouse culture maintained at 24°C. It is possible that mites exposed to ambient temperatures in the field might be better acclimated for overwinter survival as temperatures drop in the fall. Further evaluation of overwinter survival will be possible when the two overwintering studies set out in 2004 are evaluated in spring 2005.

6 Impact

This study has shown that a vigorous colony of the gall mite *C. rouhollahi* can be maintained under greenhouse conditions in Alberta, and that the mites will readily colonize false cleavers plants under field conditions. Early application of mites can lead to significant reductions in false cleavers seed production and biomass. The impact of the mite can probably be enhanced by developing application methods that distribute it evenly over a stand of false cleavers at an early growth stage, so that the whole stand becomes uniformly infested. Provided that such methods can be developed, this mite appears to have potential for control of this important weed in canola and other annual crops in Alberta.

7 Acknowledgments

I thank the Canola Agronomic Research Program for funding support, Dr. Rouhollah Sobhian for collections and shipments of mites from France, Robert B. Hughes for technical assistance, Dr. James Amrine for identification of mites, Andrea Harness (Agdia Inc.) for assistance and advice

with virus testing, Dr. Rose DeClerck-Floate, Eva Pavlik, and Dr. Rob Bourchier for quarantine assistance, John Mayko, Jennifer Otani, Alex Skorowodko, Calvin Yoder, Gary Ropchan, and the Peace Region Forage Seed Association for assistance and support for field releases, and Dr. Paul Watson and Jeff Newman for assistance with field plots at Vegreville.

8 References

Britten, D.C., G.L. Schuster, G.J. Michels, and D.A. Owings. 2003. Using cold-stored or overwintering *Aceria malherbae* Nuzzaci (Acarina: Eriophyidae), a gall-forming eriophyid mite, for infestation of field bindweed. *Southwestern Entomologist* **28**: 273-280.

Craemer, C., R. Sobhian, A.S. McClay, and J.W. Amrine. 1999. A new species of *Cecidophyes* (Acari: Eriophyidae) from *Galium aparine* (Rubiaceae) with notes on its biology and potential as a biological control agent for *Galium spurium*. *International Journal of Acarology* **25**: 255-263.

Gispert, C., G.N. Oldfield, T.M. Perring, and R. Creamer. 1998. Biology of the transmission of peach mosaic virus by *Eriophyes insidiosus* (Acari: Eriophyidae). *Plant Disease* **82**: 1371-1374.

Hall, L.M., K.M. Stromme, G.P. Horsman, and M.D. Devine. 1998. Resistance to acetolactate synthase inhibitors and quinclorac in a biotype of false cleavers (*Galium spurium*). *Weed Science* **46**: 390-396.

Harvey, T.L., and D.L. Seifers. 1991. Transmission of wheat streak mosaic virus to sorghum by the wheat curl mite (Acari: Eriophyidae). *Journal of the Kansas Entomological Society* **64**: 18-22.

Kulkarni, N.K., P.L. Kumar, V. Muniyappa, A.T. Jones, and D.V.R. Reddy. 2002. Transmission of Pigeon pea sterility mosaic virus by the eriophyid mite, *Aceria cajani* (Acari: Arthropoda). *Plant Disease* **86**: 1297-1302.

Leeson, J.Y., A.G. Thomas, and L.M. Hall. 2002. *Alberta Weed Survey of Cereal, Pulse, and Oilseed Crops in 2001, Weed Survey Series Publication 02-1*: Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan. 263 pp.

McClay, A.S., R. Sobhian, C. Craemer, and M. Peterschmitt. 2001. Petition for field release of the gall mite *Cecidophyes rouhollahi* Craemer (Acari: Eriophyidae) from southern France for biological control of false cleavers, *Galium spurium* L. (Rubiaceae), in western Canada. Vegreville, Alberta: Alberta Research Council.

Michels, G.J., D.A. Fritts, and J.B. Bible. 1999. Release and colonization of the bindweed gall mite, *Aceria malherbae*: a field bindweed biological control program for the Texas High Plains. pp. 140-141 *in* Spencer, N.R. (Ed.) Proceedings of the X International Symposium on Biological Control of Weeds, Bozeman, Montana. USDA-ARS.

Moha, C. 1972. Sur la présence d'inclusions de type particulier dans les cellules qui bordent la cavité gallaire de la cécidie d'*Eriophyes galii* Karp sur *Galium aparine* L. *Journal de Microscopie* **14**: 70a-71a.

Sobhian, R., A. McClay, S. Hasan, M. Peterschmitt, and R.B. Hughes. 2004. Safety assessment and potential of *Cecidophyes rouhollahi* (Acari, Eriophyidae) for biological control of *Galium spurium* (Rubiaceae) in North America. *Journal of Applied Entomology* **128**: 258-266.

Thomas, A.G., and R.F. Wise. 1985. *Dew's Alberta Weed Survey 1973-1977, Weed Survey Series Publication 85-3*: Agriculture Canada, Regina, SK. 134 pp.

Thomas, A.G., B. Frick, and L. Hall. 1998a. Weed population shifts in Alberta. Saskatoon: Agriculture and Agri-Food Canada.
Thomas, A.G., B. Frick, R. van Acker, and D. Joosse. 1998b. Weed population shifts in Manitoba. Saskatoon: Agriculture and Agri-Food Canada.
Thomas, A.G., B. Frick, R.F. Wise, and L.T. Juras. 1998c. Weed population shifts in Saskatchewan. Saskatoon: Agriculture and Agri-Food Canada.

Table 1. Effect of false cleavers on canola seed yield. Field plot experiment, Vegreville, 2003.

	With false cleavers	Without false cleavers
Canola seed yield (g m^{-2}) (mean ± S.E.)	151.5 ± 6.3	163.6 ± 6.6

Table 2. False cleavers stem and leaf biomass and seed production in plots with and without canola. Field plot experiment, Vegreville, 2003.

	With canola	Without canola
False cleavers seed weight (g m^{-2})	1.2 ± 0.2	161.1 ± 11.5
False cleavers stem and leaf biomass (g m^{-2})	3.9 ± 0.4	242.0 ± 13.2

Table 3. Effects of mite inoculation on false cleavers seed yield, stem and leaf biomass, gall ratings, and canola seed yield in plots with canola. Field plot experiment, Vegreville, 2003.

Mites applied	False cleavers seed weight (g m^{-2})	False cleavers stem and leaf biomass (g m^{-2})	Gall damage in September	Canola seed yield (g m^{-2})
early	0.93 ± 0.39	3.14 ± 0.76	0.00 ± 0.15	155.5 ± 11.1
late	1.16 ± 0.39	3.83 ± 0.76	0.67 ± 0.15	145.7 ± 11.1
none	1.43 ± 0.39	4.60 ± 0.76	0.00 ± 0.15	153.4 ± 11.1

Table 4. Effects of mite inoculation on false cleavers seed yield, stem and leaf biomass, and gall ratings in plots without canola. Field plot experiment, Vegreville, 2003.

Mites applied	False cleavers seed weight (g m^{-2})	False cleavers stem and leaf biomass (g m^{-2})	Gall damage in September	Canola seed yield (g m^{-2})
early	152.2 ± 19.9	245.0 ± 23.4	0.33 ± 0.20	N/A
late	170.6 ± 19.9	249.0 ± 23.4	1.50 ± 0.20	N/A
none	160.5 ± 19.9	231.9 ± 23.4	0.00 ± 0.20	N/A

Table 5. Least-square estimates of false cleavers biomass, seed yield and seed ratio in plots with and without canola, Vegreville, 2004. Values within rows followed by different letters are significantly different at $p<0.05$, Tukey's HSD test.

	Without canola	With canola
False cleavers aboveground biomass (g m^{-2})	313.15 A	50.99 B
False cleavers seed yield (g m^{-2})	125.18 A	20.09 B
False cleavers seed ratio	0.4042 A	0.4272 A

Table 6. Analysis of variance for false cleavers total aboveground biomass, untransformed data, Vegreville field plot experiment 2004. Note: SS are marginal (type III) sums of squares.

Source of variation	DF	Sum of Squares	MS	F	p	
Main plot	11	162398	14763			
Mites	2	47508	23754	3.52	0.0368	*
Canola	1	1179769	1179769	174.73	0.0000	***
Mites*Canola	2	23381	11690	1.73	0.1869	NS
Error	53	357848	6752			
Total	69					

Table 7. Analysis of variance for false cleavers seed weight, untransformed data, Vegreville field plot experiment 2004. Note: SS are marginal (type III) sums of squares.

Source of variation	DF	Sum of Squares	MS	F	p	
Main plot	11	20752	1887			
Mites	2	9933	4966	4.47	0.0160	*
Canola	1	193132	193132	173.79	0.0000	***
Mites*Canola	2	5201	2600	2.34	0.1060	
Error	54	60011	1111			
Total	70					

Table 8. Results of cold temperature storage experiment. “+” indicates successful gall initiation by mites after storage at the indicated temperature. “-” indicates no gall initiation.

Temperature °C		Days storage									
Nominal	Actual	1	2	3	4	7	10	14	17	21	24
4	4.2	+	+	+	+	+	+	+	+	+	+
0	0.5	+	+	+	+	+	+	+	+	-	+
-5	-4.2	+	+	+	+	+	+	+	+	+	+
-10	-8.0	+	+	+	+	+	+	+	+	+	+
-15	-15.4	+	+	-	-	-	-	-	-	-	-
-20	-20.0	+	+	-	-	-	-	-	-	-	-
-25	-25.0	-	-	-	-	-	-	-	-	-	-
-80	-80.0	-	+	-	-	-	-	-	-	-	-

Figure 1. Technique for inoculation of gall mites onto false cleavers seedlings in field plots.

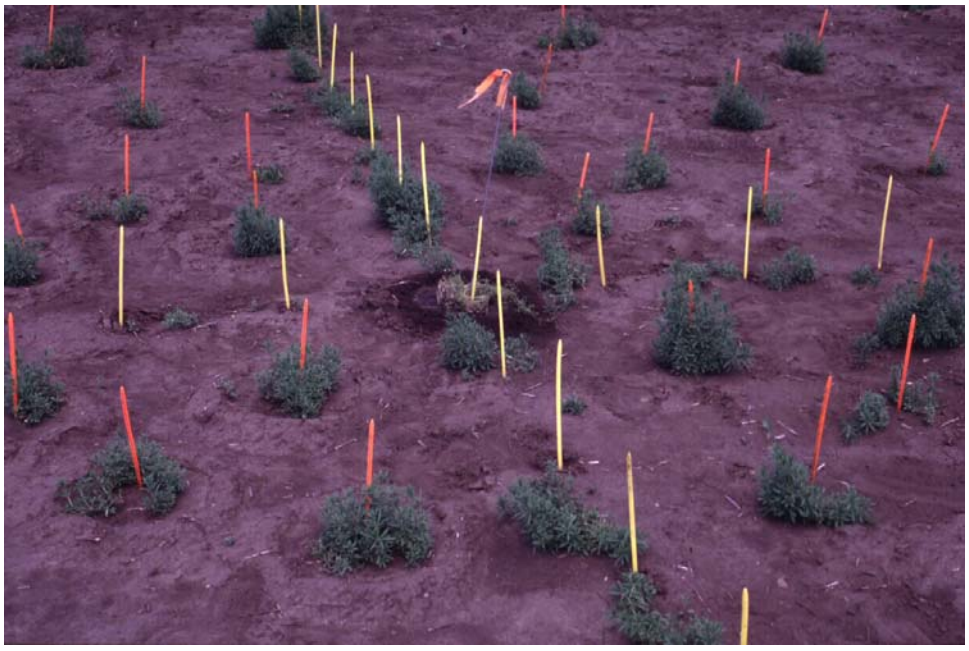

Figure 2. Gall mite dispersal field experiment, Vegreville 2003.

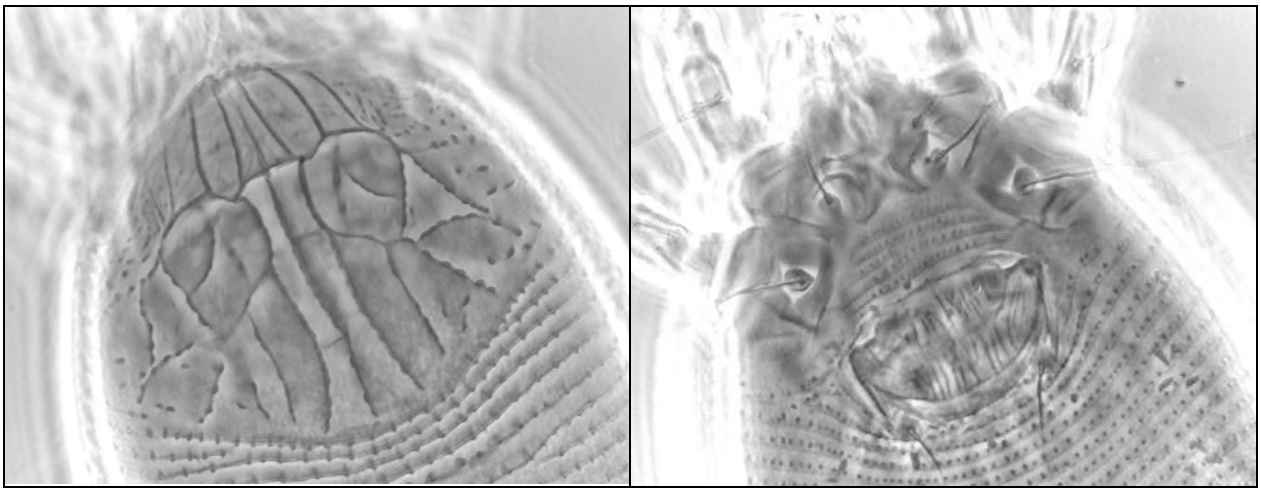

Figure 3. Photomicrographs of mites from 2002 shipment, confirming identification as *Cecidophyes rouhollahi*. Left, dorsal shield; right, female coxal-genital region. Courtesy of Dr. J.W. Amrine, West Virginia State University.

Figure 4. Shoot tips of false cleavers showing gall damage caused by *Cecidophyes rouhollahi*

Figure 5. Stem discolouration developing on heavily mite-infested false cleavers plant.

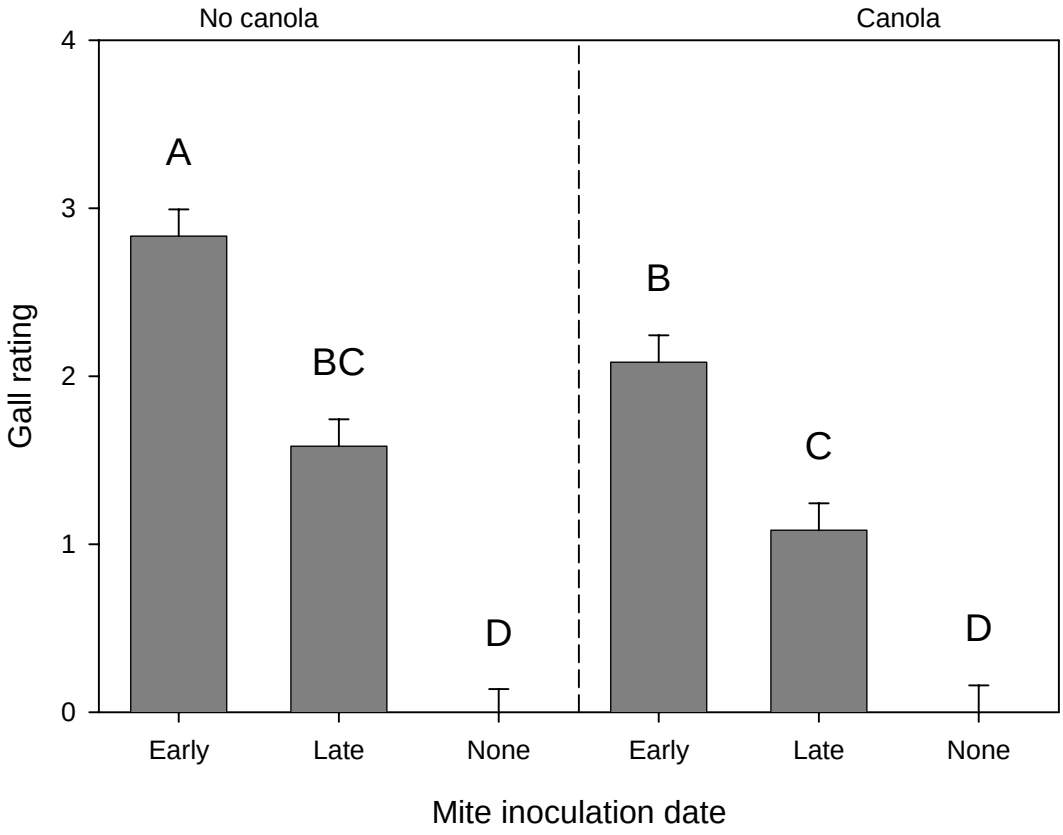

Figure 6. Effect of mite inoculation date and presence or absence of canola on gall damage ratings on false cleavers on August 4, 2004. Columns with different letters are significantly different at $p<0.05$, Tukey's HSD test.

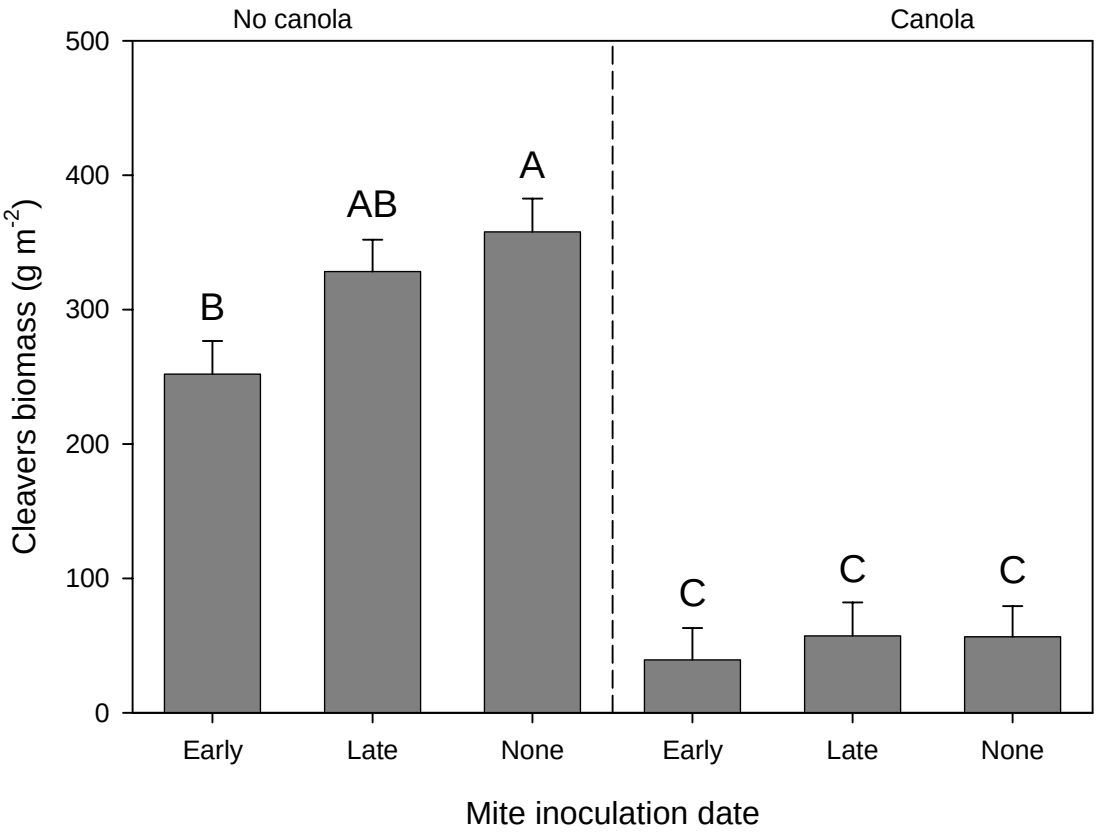

Figure 7. Effects of mite inoculation date on false cleavers biomass in plots with and without canola, Vegreville field plot experiment, 2004. Columns with different letters are significantly different, $p<0.05$, Tukey's HSD test.

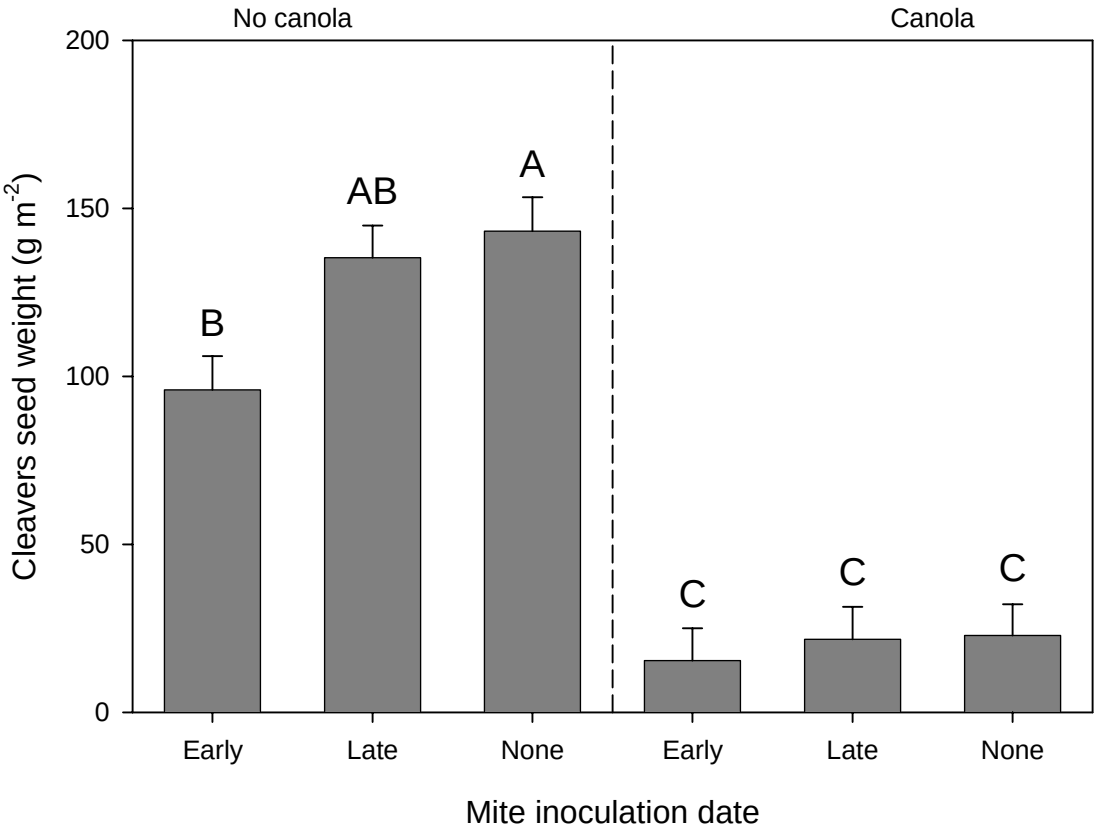

Figure 8. Effects of mite inoculation date on false cleavers seed weight in plots with and without canola, Vegreville field plot experiment, 2004. Columns with different letters are significantly different, $p<0.05$, Tukey's HSD test.

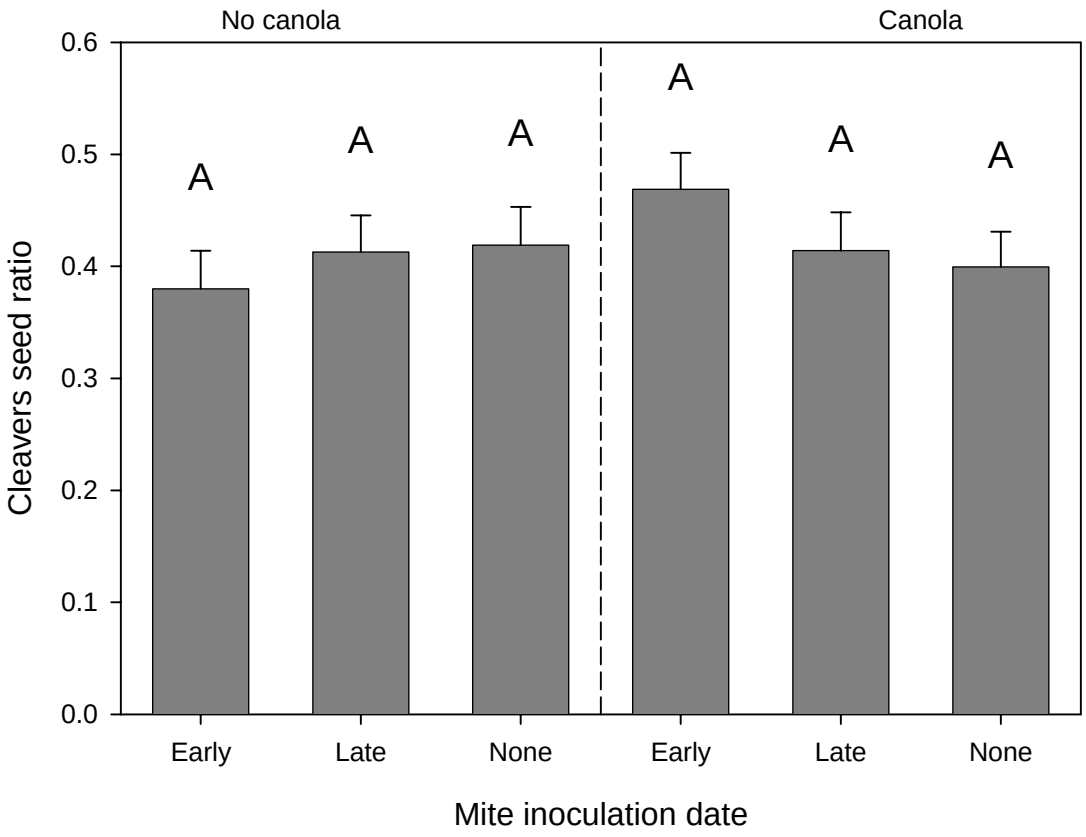

Figure 9. Effects of mite inoculation date on false cleavers seed ratio (proportion of aboveground biomass allocated to seed production) in plots with and without canola, Vegreville field plot experiment, 2004. Columns with different letters are significantly different, $p<0.05$, Tukey's HSD test.

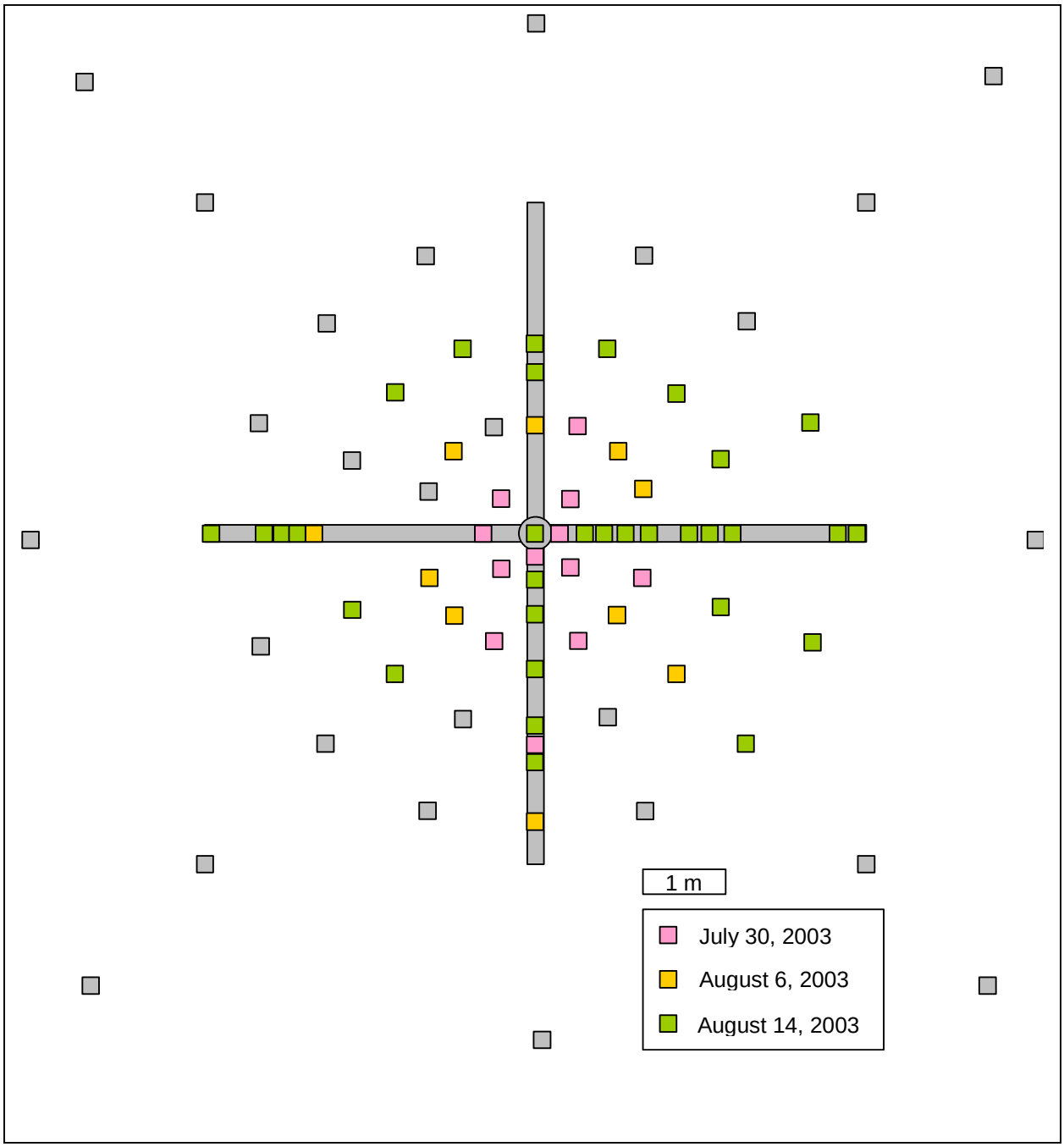

Figure 10. Results of dispersal experiment with *Cecidophyes rouhollahi*, Vegreville, 2003. Grey strips and squares indicate strips or isolated plants of false cleavers, respectively. Colours indicate dates on which galling was first found at each location. Scale bar is 1 m.

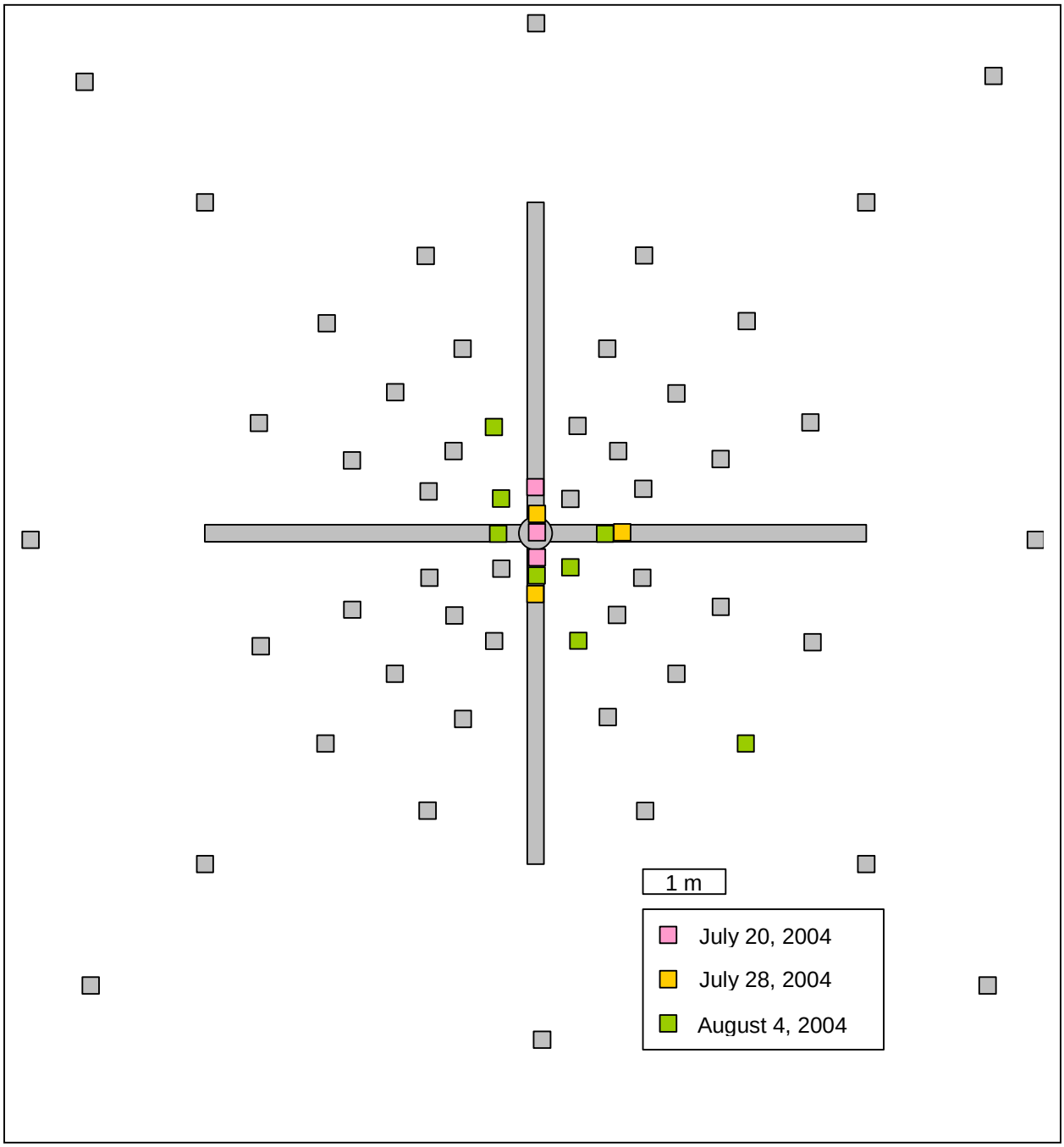

Figure 11. Results of dispersal experiment with *Cecidophyes rouhollahi*, Vegreville, 2004. Grey strips and squares indicate strips or isolated plants of false cleavers, respectively. Colours indicate dates on which galling was first found at each location. Scale bar is 1 m.

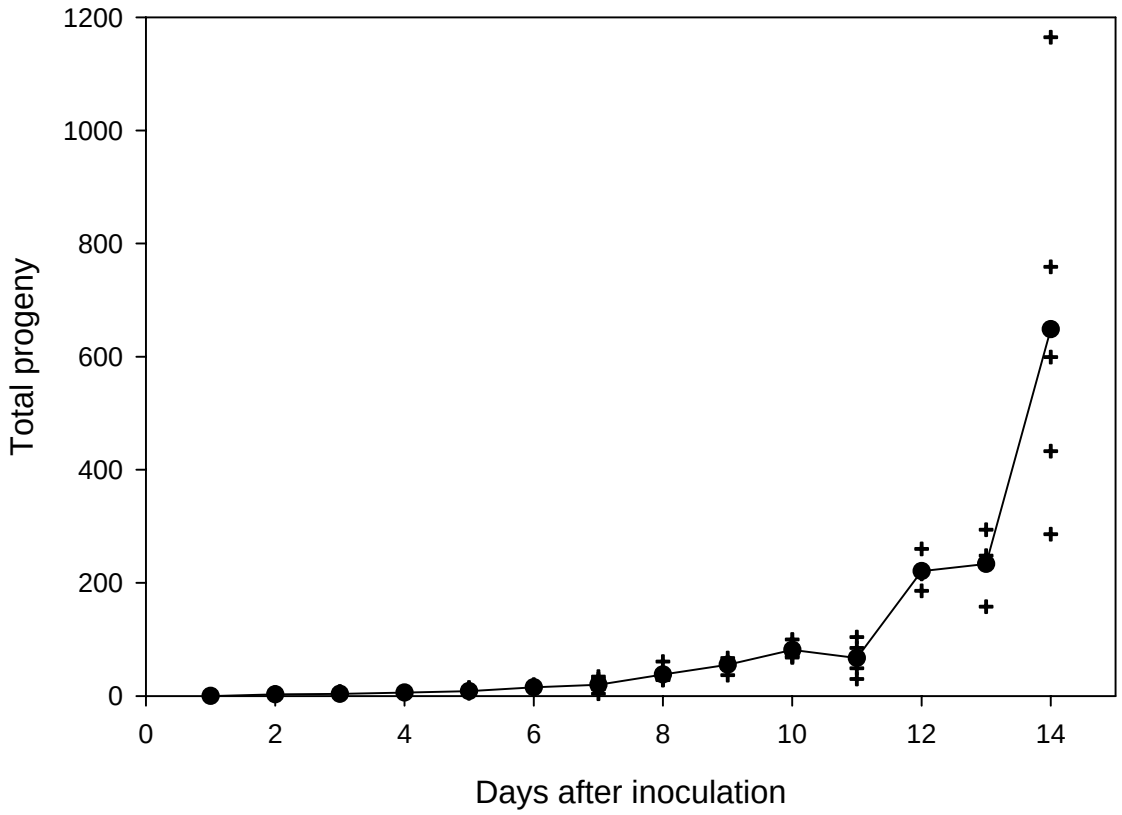

Figure 12. Numbers of descendents produced after 1 – 14 days by a single female *C. rouhollahi* mite placed on a cleavers seedling at constant 25°C. "+" signs indicate individual replicates, black circles indicate means.

APPENDIX

Letter from Dr. A. Cofrancesco, Chair, USDA-APHIS Technical Advisory Group on Biological Control Agents of Weeds, dated December 12, 2001, recommending approval for the release of *Cecidophyes rouhollahi*.

Letter from Dr. Y. Singh, Acting Director, Plant Products Directorate, Canadian Food Inspection Agency, dated June 6, 2002, approving release of *Cecidophyes rouhollahi* in the field in Canada.

DEPARTMENT OF THE ARMY
ENGINEER RESEARCH AND DEVELOPMENT CENTER, CORPS OF ENGINEERS
ENVIRONMENTAL LABORATORY
WATERWAYS EXPERIMENT STATION, 3909 HALLS FERRY ROAD
VICKSBURG, MISSISSIPPI 39180-6199

December 12, 2001

COPY RETAINED

REPLY TO
ATTENTION OF:

Environmental Laboratory

Ms. Polly Lehtonen
USDA, APHIS, BATS
4700 River Road
Unit 133
Riverdale, Maryland 20737-1236

Dear Polly:

I have reviewed the fourteen comments for Petition Number 01-01, "Petition for Field Release of the Gall Mite *Cecidophyes rouhollahi* Craemer (Acari: Eriophyidae) from southern France for Biological Control of False Cleavers, *Galium spurium* L. (Rubiaceae), in western Canada."

Eight individuals recommended release of the agent without reservations, five reviewers recommended release with reservations, while one individual recommended not to release. The petition has raised some questions about the ability of the agent to transmit a virus. Other minor problems have also surfaced during the review. One reviewer expressed concern over the non-target impact to *Gallium aparine* while another seemed concerned on the sample size use in testing. In individual discussions conducted with many of the reviewers they feel that the research was done carefully and that the results presented show a safe host-specific agent that will impact *G. spurium* L.

The TAG recommends that the gall mite *C. rouhollahi* Craemer be approved for use as a biological control agent of *G. spurium* L. However, it is recommended that the researchers examine the concerns expressed by all reviewers and consider them as they develop a protocol for release and monitor the impact of the agent. I am enclosing the comments of each individual for your records. I hope that this information will assist the researcher in conducting the release and establishment of field populations.

Sincerely,

Alfred F. Cofrancesco, PhD
Chairman, Technical Advisory Group
U.S. Army Corps of Engineers

Copies Furnished: TAG Members

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Plant Products Directorate
Plant Health and Production Division
Canadian Food Inspection Agency
Nepean, Ontario
K1A 0Y9
Phone: (613) 225-2342
Fax: (613) 228-6602

June 6, 2002

Dr. A. S. McClay
Alberta Research Council
PO Bag 4000
Vegreville AB
T9C 1T4

Dear Dr. McClay,

We have reviewed your petition for the release of *Cecidophyes rouhollahi* Craemer (ACARI: Eriophyidae) for control of false cleavers, *Galium spurium* L. in nature. On the basis of the comments of the AAFC Biological Control Review Committee (BCRC), the Technical Advisory Group of USDA-APHIS and our regulatory entomologists, we hereby authorize the release of this mite in Canada.

This release pertains only to mites from the tested European population. Please inform the Entomology Laboratory of the Centre for Plant Quarantine Pests as to the exact locations and dates of releases for inclusion in the annual reports of biocontrol liberations in Canada.

If you require further information, please do not hesitate in contacting us.

Sincerely,

Dr. Yudi Singh
A/Director

cc. P. Mason, BCRC
Permit Section, CFIA
Gill/Parker, CPQP-Ent

Canada