Life cycle of *Puccinia acropilitii* on *Rhaponticum (= Acroptilon) repens*

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**Abstract:** Russian knapweed, *Rhaponticum repens* (L.) Hildago (Hildago et al. 2006; = *Acroptilon repens* (L.) DC; Asteraceae), is a perennial weed introduced into North America from central Asia. It now is reported from the Pacific Coast of North America east to Ontario, Canada, and in continental USA to Ohio, Kentucky, Arkansas and Texas (USDA NRCS 2008). It is listed as a pest in 18 states, including Alaska and Hawaii, and infests pastures, rangelands and other dry habitats (DiTomaso and Healy 2007). Because of its widespread distribution and the difficulties in management with conventional control strategies *R. repens* has been subject of research about the potential for biological control (Bruckart et al. 2005, Kim and Mortensen 1986, Mortensen et al. 1991).

*Puccinia acropilitii* Syd. & P. Syd. is a rust fungus (Pucciniaceae) that parasitizes *R. repens* in its native range in Eurasia. It is widespread also in North America (Bruckart et al. 2006, Cummins 1979, Dugan and Carris 1992, Mortensen and Molloy 1989, Palm and Vesper 1991, Savile 1970b). Based on two-dimensional polypeptide mapping *P. acropilitii* is distinct from other similar rusts on Cardueae in North America, including *P. jaceae* Otth and *P. centaureae* DC. on *Centaurea* spp., and *P. corthami* Corda on safflower (Kim and Mortensen 1986). An aggressive isolate of *P. acropilitii* from Turkey was evaluated recently as a candidate for biological control of *R. repens* (Bruckart et al. 2005), and comparisons with North American and other Eurasian isolates were initiated.

The life cycle of *P. acropilitii* has not been documented, but it is generally considered autecious. Spermogonia were observed only recently in the field (Mortensen and Molloy 1989), but they were not described. Savile (1970b) noted that “pycnia are unknown”; Cummins (1979) stated that “pycnia and acia are unknown”; and Sydow and Sydow (1904) and Wei and Wang (1986) did not mention spermogonia in their descriptions. Aecia have not been reported or described, and there is no report of attempted crosses or functionality of different spore types, with the exception of urediniospores (Mortensen et al. 1991).

Knowledge of the life cycle of candidate organisms is imperative for identifying sound biological control agents. Thus the objective of this study was to determine the life cycle of *P. acropilitii* based on artificial inoculations under controlled greenhouse conditions.

**INTRODUCTION**

Russian knapweed, *Rhaponticum repens* (L.) Hildago (Hildago et al. 2006; = *Acroptilon repens* (L.) DC; Asteraceae), is a perennial weed introduced into North America from central Asia. It now is reported from the Pacific Coast of North America east to Ontario, Canada, and in continental USA to Ohio, Kentucky, Arkansas and Texas (USDA NRCS 2008). It is listed as a pest in 18 states, including Alaska and Hawaii, and infests pastures, rangelands and other dry habitats (DiTomaso and Healy 2007). Because of its widespread distribution and the difficulties in management with conventional control strategies *R. repens* has been subject of research about the potential for biological control (Bruckart et al. 2005, Kim and Mortensen 1986, Mortensen et al. 1991).

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Knowledge of the life cycle of candidate organisms is imperative for identifying sound biological control agents. Thus the objective of this study was to determine the life cycle of *P. acropilitii* based on artificial inoculations under controlled greenhouse conditions.
with wetting agent, as described, and applied either by suspension onto plants. Inoculated plants were incubated in a dew chamber at 18°C for 72 h with 16 h dark followed by constant light, thus triggering development of basidia and basidiospores on the leaves. Plants were placed on a bench in a cubic in the containment greenhouse at 20°C with supplemental lighting as described. Plants were observed for symptoms, and data on sori type and quantity were collected.

**CROSSES.**—Leaves were inoculated with teliospores to produce spermogonia and spermatia for crossing experiments. When flecks appeared leaves were detached and leaf bases inserted through Parafilm® (Pechiney Plastic Packaging, Menasha, Wisconsin) into jars of sterile tap water (Fig. 1). Jars were put in insect exclusion cages to prevent uncontrolled crossing between spermogonia by shore flies (Scactella stagnalis [Fallen]) and fungus gnats (Lycoriella spp. and Bradyia spp.). Self-crosses were made with six of the isolates that included both USA and Eurasian acces-
sions. Spermogonia from each isolate were collected by excising a single, isolated, orange spermogonium from a leaf, washing it in 50 μL sterile distilled water and transferring 1 μL drops suspension to each of the remaining spermogonia of the same isolate. Transfers were made with a P2 Gilson/Rainin Pipetman® (Gilson Inc., Middleton, Wisconsin) pipette with a new tip for each spermogonial transfer. Leaves were mapped for distribution of spermogonia and treated leaves were returned to insect exclusion cages as described, and leaves were observed for development of accia. Spermogonia with accia were recorded on leaf maps, and percentage of successful crosses was calculated for each isolate.

**Measurements.**—Spores were measured with a Nikon Eclips 80i microscope equipped with a DS-L1 camera and a flat-screen monitor. Software enabled on-monitor measurement of objects in the field of view, calibrated for each level of magnification. A minimum of 50 spores was measured for each isolate and spore form. In addition urediniospores and aeciospores were measured with a microscope equipped with a 10x objective and a square test screen.

**Isolates.**—Eleven isolates of *P. acroptili* were received from Eurasia and USA as viable urediniospores and teliospores on dried leaves (Table 1). A portion of urediniospores from each original sample was stored in freezer at –80°C. Urediniospores from original samples also were used for spore increase through artificial inoculation, and greenhouse-produced urediniospores were stored either at –80°C or 4°C. All teliospores, whether from original samples or from greenhouse production, were stored on dried-leaf material, either under refrigeration, some at very low temperatures.

**Inoculations.**—Inoculations were made most frequently with urediniospores or teliospores, but inoculation either with aeciospores or spores that developed in aecium-like sori after teliospore inoculations was made in a few cases to confirm viability and pathogenicity. Sori were suspended in distilled water plus a wetting agent (0.15% v/v Tween® 20 [polyoxyethylene sorbitan monolaurate]) and sprayed onto 4 wk old potted *R. repens* plants at 0.5 mg/plant. Plants were given two 16 h dew treatments at 18°C in the dark, with 8 h daylight between dew periods. Inoculated plants were removed from the dew chamber, bench in a containment greenhouse at 21–25°C with supplemental lighting to maintain a 14 h photoperiod and observed for infection.

Teliospores for inoculation were primed 1 wk by placing stems and leaves with telia in a Petri dish containing moist towels and incubating them under refrigeration. After priming ungerminated teliospores were suspended in water with wetting agent, as described, and applied either by finger to individual leaves or by spraying the teliospore suspension onto plants. Inoculated plants were incubated in a dew chamber at 18°C for 72 h with 16 h dark followed by constant light, thus triggering development of basidia and basidiospores on the leaves. Plants were placed on a bench in a cubic in the containment greenhouse at 20°C with supplemental lighting as described. Plants were observed for symptoms, and data on sori type and quantity were collected.

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teliospores were measured from two herbarium specimens collected in New Mexico and borrowed from the U.S. National Fungus Collections in Beltsville, Maryland (BPI 1107952 and 1110177, Table I).

Spores were mounted on slides with a drop of 1% aniline blue in lactophenol, and each slide was heated gently until spores were turgid (Savile 1970a). Urediniospores, acerciospores, and spores developing in the acercium-like sori following teliospore inoculations were measured both for length and width, and the average of these measurements was used for data analysis. Teliospores and spermatia were measured for length and width, and data for each of these dimensions were used directly in statistical analyses. Cross-sections of spermagonia, stained with lactophenol and aniline blue, also were measured as described.

Statistical treatments.—Calculation of means and confidence intervals ($P = 0.05$) were made with Microsoft® Office Excel 2003 software. Data either were pooled for all isolates or analyzed as the mean of isolate means. Results are presented as mean ($\pm$ confidence interval).

RESULTS

All spore types were observed on leaves and stems of inoculated Russian knapweed plants except basidiiospores, which were seen during microscopic examination of germinating teliospores on water agar. All tested spore forms were functional, resulting in completion of the life cycle of *P. aero/itii* under greenhouse conditions.

Urediniospore inoculations.—Inoculations with urediniospores resulted in uredinia typical for *Puccinia* species on plants in tribe Cardueae (Fig. 2, left). Sori were brown with no associated hypertrophic leaf tissue. Urediniospores were golden brown and generally oval, had three more-or-less equatorial germ pores and were $22.1(\pm 0.4) \mu m$ diam ($n = 10$ isolates, Table II).

Telia and teliospores.—Telia developed eventually from urediniospore inoculations. Sori were dark brown with no surrounding hypertrophic leaf tissue. Teliospores were typically dark brown, 1-septate, with a distinct pedicel (Fig. 3). The average width $\times$ length for teliospores from 12 isolates was $23.1(\pm 0.4) \times 38.1(\pm 0.8) \mu m$ (Table II). Germinating telio-

Table II. Dimensions (\(\mu m\)) of *Puccinia aero/itii* spores from different sources (\(n \geq 50\) for each isolate)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Telia Width</th>
<th>Telia Length</th>
<th>Urediniaa</th>
</tr>
</thead>
<tbody>
<tr>
<td>90-114</td>
<td>22.2</td>
<td>39.3</td>
<td>22.4</td>
</tr>
<tr>
<td>01-995</td>
<td>23.0</td>
<td>38.5</td>
<td>22.7</td>
</tr>
<tr>
<td>02-048</td>
<td>22.0</td>
<td>41.2</td>
<td>22.4</td>
</tr>
<tr>
<td>05-051</td>
<td>22.7</td>
<td>38.0</td>
<td>22.5</td>
</tr>
<tr>
<td>05-052</td>
<td>23.6</td>
<td>38.1</td>
<td>21.6</td>
</tr>
<tr>
<td>05-053</td>
<td>23.3</td>
<td>37.3</td>
<td>22.1</td>
</tr>
<tr>
<td>05-054</td>
<td>24.0</td>
<td>37.5</td>
<td>nd</td>
</tr>
<tr>
<td>05-055</td>
<td>22.2</td>
<td>38.1</td>
<td>21.2</td>
</tr>
<tr>
<td>05-056</td>
<td>23.4</td>
<td>36.8</td>
<td>21.8</td>
</tr>
<tr>
<td>05-069</td>
<td>23.5</td>
<td>36.6</td>
<td>21.4</td>
</tr>
<tr>
<td>05-070</td>
<td>23.0</td>
<td>35.9</td>
<td>23.3</td>
</tr>
<tr>
<td>05-085</td>
<td>24.7</td>
<td>39.7</td>
<td>nd</td>
</tr>
<tr>
<td>Mean (ci$^b$)</td>
<td>23.1(0.4)</td>
<td>38.1(0.8)</td>
<td>22.1(0.4)</td>
</tr>
</tbody>
</table>

*a* Average of width and length.

$^b$ ci = confidence interval for the mean of the means ($P = 0.05$).
spores produced basidia with four basidiospores per basidium on water agar.

**Amphispores and Mesospores.**—Amphispores (Fig. 3A) and mesospores (Fig. 3B-D) were noted infrequently in greenhouse samples of urediniospores and teliospores respectively. Amphispores were similar in color and morphology to urediniospores, but they were significantly larger and had thick walls (Kirk et al 2001). Amphispores were 28.7(± 0.7) μm diam (n = 13). Mesospores were 1-celled teliospores produced among 2-celled teliospores (Kirk et al 2001), and they were 24.6(± 1.6) × 32.1(± 1.7) μm (n = 11).

**Teliospore inoculations.**—Inoculations with primed, ungerminated teliospores placed directly on plant leaves resulted in development of two types of sori, yellow-orange spermogonial sori and accium-like sori (Fig. 2, right). Orange aggregations of spermogonia were evidence of basidiospore infections and appeared on hypertrophic leaf tissue surrounding the sori. However accium-like sori developed in greater proportion than spermogonia after teliospore inoculations (Fig. 2, right). Accium-like sori were produced by all six isolates tested, and they occurred 1.8 times more frequently than spermogonia for three isolates, 02-048, 05-085 and 05-055, for which data were collected. These sori, which were surrounded by hypertrophic leaf tissue, were not associated with spermogonia.

**Spores from accium-like sori.**—Accium-like sori resulted directly from teliospore inoculations and generated spores that were morphologically similar to aeciospores. Mean diameter of spores from accium-like sori of three isolates (TABLE III) was 24.8(± 1.1), which is significantly larger than those of normal urediniospores (TABLE II) but not different from aeciospores (TABLE III).

**Spermogonia and spermatia.**—Spermogonia generally were aggregated into yellow-orange sori (Fig. 4) that were covered with a sugary matrix of spermatia. Individual spermogonia were flask-shape with flexuous hyphae extending from an ostiole (Figs. 5, 6). For isolate 02-048 spermogonia were 127.5 ± 10.9 μm diam (n = 25) and were classified as typical *Puccinia* Group V, Type 4 spermogonia (Hiratsuka and Cummins 1963). Spermatia were oval, hyaline and small at 2.1(± 0.1) × 3.3(± 0.2) μm wide and long respectively for isolates 02-048 and 05-055 (TABLE III).

**Fertilization of spermogonia; accia and aeciospores.**—Accia developed from self crosses within (each of the six isolates (TABLE IV, Figs. 4, 6). No insect activity was detected inside cages, so both successful and unsuccessful crosses resulted from the artificial transfer of spermatia. Accia were similar to uredinia,

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Spermogonium</th>
<th>AL</th>
<th>Aecium</th>
<th>Urediniospores from inoculation by spores from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Source of spores</td>
<td></td>
<td></td>
<td>AL</td>
</tr>
<tr>
<td>02-048</td>
<td>2.1 × 3.1</td>
<td>24.0</td>
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<td>nd</td>
<td>nd</td>
<td>25.3</td>
<td>22.8</td>
</tr>
<tr>
<td>06-037</td>
<td>nd</td>
<td>26.1</td>
<td>25.2</td>
<td>nd</td>
</tr>
<tr>
<td>Mean (ci)</td>
<td>(see text)</td>
<td>24.8(1.1)</td>
<td>24.5(0.9)</td>
<td>22.8(0.5)</td>
</tr>
</tbody>
</table>

* Sori from teliospore inoculations that are accium-like and not the result of a cross between compatible spermogonia.
* nd = not determined.
* ci = confidence interval for the mean of the means (P = 0.05).
FIG. 4. Four clusters of spermogonia, each inoculated with spermatia from a single sorus. The two orange soroi on either end were not fertilized and aecia did not develop after the transfer of spermatia. Two soroi between the orange unfertilized soroi are ringed by aecia, results of compatible crosses. Insert: A cluster of spermogonia with a ring of aecia.

but they either developed within or on the margins of spermogonial clusters (Fig. 4) or they emerged from the opposite surface of the leaf from spermogonia (Fig. 6). Aeciospores were also similar to urediniospores (i.e. uredinoid aeciospores) in gross morphology, but they, like spores from the aecium-like soroi, were significantly larger (TABLE II) than urediniospores (TABLE II). Mean aeciospore diameter from four isolates was 24.5(± 0.9) (TABLE III).

Plant inoculations either with aeciospores or spores from aecium-like soroi.—Inoculations using either of these spores resulted in development of uredinia (i.e. soroi with no associated hypertrophic growth and producing the dikaryotic repetitive spore stage). Urediniospores from these soroi were the same size (TABLE III) as other urediniospores in the study (TABLE II).

DISCUSSION

Results of these investigations indicate that P. acroptili is autecious and macrocyclic. All known spore forms of Puccinia were observed and found functional. Sizes of both urediniospores and teliospores were within the ranges described by others for P. acroptili (Bruckart et al 2006, Cummins 1979, Dugan and Carris 1992, Mortensen and Molloy 1989, Palm and Vesper 1991, Savile 1970b, Sydow and Sydow 1904, Wei and Wang 1986). Basidiospores, spermogonia and spermatia, aecia and aeciospores, amphispores and mesosporcs had not been described before this study, although spermogonia were reported by Mortensen and Molloy (1989) from field observations.

Production of aecium-like soroi directly from inoculation with teliospores was not expected and is reported herein also for the first time. These soroi were similar to aecia in color and symptomatology, including hypertropic growth of plant tissue surrounding soroi, but they were not associated with spermogonia or derived from any observed classical fertilization. Position in the life cycle (i.e. development following teliospore inoculations) and the associated hypertrophic leaf tissue distinguished them from uredinia. Spores from these soroi were the same size and shape as those produced in aecia, and when these were inoculated onto plants uredinia developed as would be expected after inoculation by aeciospores. Ontogeny and karyogamy of the spores produced in aecium-like soroi is not known.

At least two examples have been reported of aecial
development without pycnia. These concern *Puccinia allii* (DC) Rud. (Anikster et al 2004) and *P. coronata* Corda f. sp. *bromi* sensu Mühlethalera (Anikster et al 2003). In each of these cases the variant of the fungus produced only two spores per basidium and the nuclear condition of each basidiospore was double that of typical basidiospores. These two cases differ because they were cultivar-specific, occurred in mixtures rarely. 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