Artificial Inoculation and Colonization of Dyer's Woad (*Isatis tinctoria*) by the Systemic Rust Fungus *Puccinia thlaspeos*

Bradley R. Kropp, Dane Hansen, Karen M. Flint, and Sherman V. Thomson

Department of Biology, Utah State University, Logan, 84322.
This research was supported by the Utah Department of Agriculture and the USDA Rangeland Research Program.
Utah Agricultural Experiment Station paper 4872.
Accepted for publication 23 May 1996.

ABSTRACT

Kropp, B. R., Hansen, D., Flint, K. M., and Thomson, S. V. 1996. Artificial inoculation and colonization of dyer's woad (*Isatis tinctoria*) by the systemic rust fungus *Puccinia thlaspeos*. Phytopathology 86:891-896.

Dyer's woad plants were inoculated successfully with the systemic rust fungus, *Puccinia thlaspeos*, using a dew chamber. At least 94% of plants became diseased when whole plants were inoculated, but only 28% became diseased when single leaves were inoculated. The plants were asymptomatic for at least 10 weeks after inoculation, but some remained asymptomatic for as long as 9 months. Polymerase chain reaction was used to study asymptomatic colonization by the rust. After inoculation at the leaf tip, *P. thlaspeos* moved through the leaf at a rate of about 0.25 cm per week. By 10 weeks, the fungus had grown down the leaf, through the petiole, and into new leaves produced by the plant. The fungus was detected in most leaves and in the roots of an overwintering rosette that was infected, but asymptomatic. *P. thlaspeos* likely invades woad through its leaves and moves into the meristematic areas and roots where it overwinters. Second-season plants, including the roots and asymptomatic shoots, were completely colonized by *P. thlaspeos* after bolting. Seed produced by diseased plants germinated as well as seed from controls.

Additional keywords: biological control, noxious weed, PCR.

Dyer's woad (*Isatis tinctoria* L.) is a biennial crucifer that was cultivated in Europe for centuries as a source of blue dye. It was introduced into eastern North America as a dye crop by the early colonists (14) and may have been brought to the western states in the early 1900s as a dye crop or as a contaminant in alfalfa (*Medicago sativa* L.) seed (3). Although dyer's woad is not considered to be a problem in the eastern part of the continent, it has become a noxious weed in the western states.

Woad is an aggressive colonizer, capable of outcompeting native vegetation in the semiarid west by virtue of its drought tolerance and prolific seed production (3,4). Its seed are also thought to contain allelopathic compounds that help it compete with neighboring plants (15). Range cattle, sheep, and deer all appear to avoid grazing on it. Large populations of woad can now be found in crop and rangeland in the intermountain west, particularly southern Idaho, northern Utah, and western Wyoming (3,14).

Although dyer's woad can be controlled with herbicides in agricultural areas, it is much more difficult to control in rangelands where access may be limited and large areas require treatment. In such areas, biological control measures may be the most viable option for limiting the spread of this weed. In 1978, a species of rust causing disease on dyer's woad was found in southern Idaho (12). This rust has been tentatively identified as *Puccinia thlaspeos* Schub. and is an autoecious, microcyclic species (2) that produces only spermatia and telia on dyer's woad. Because the rust fungus causes systemic infections and reduces seed production, it limits the spread of dyer's woad, and biological control appears to be a realistic strategy for checking the spread of this weed.

Dyer's woad most often has a biennial life cycle. The rosette formed during the first growing season overwinters and bolts the following spring to form a flower stalk and seed by the end of the second season. *P. thlaspeos* usually appears to infect the plants during the early part of the first growing season, but the infected plants remain asymptomatic for 3 to 9 months afterwards (5,10). Thus, infected rosettes usually do not develop symptoms until the spring of the second year. The first symptoms appear on the rosette as leaf chlorosis and distortion, followed by the production of spermatia and telia. Bolting plants produce stunted stalks that are distorted and usually fail to produce flowers and seed. Occasionally, some infected symptomatic plants produce a shoot that appears normal with flowers and seed. It was unknown if these plants escaped infection or were infected asymptomatically.

Little is known about the process by which *P. thlaspeos* infects and colonizes dyer's woad. Previous attempts to infect woad plants under artificial conditions were unsuccessful (5). Since infected dyer's woad often remains asymptomatic until the beginning of the second growing season, the colonization process has been difficult to study. The objectives of the present work were to determine methods and the conditions necessary to artificially induce systemic infections of dyer's woad by *P. thlaspeos*, elucidate the process by which initial infection of dyer's woad occurs, and characterize the colonization of mature plants by *P. thlaspeos*.

MATERIALS AND METHODS

Growth of dyer's woad. Seed of dyer's woad was collected from plants growing in northern Utah and removed from the silicles to allow germination. The seed were planted into 10-cm pots containing a potting mix consisting of two parts peat moss and one part Kidman fine sandy loam that had been steam-pasteurized. The plants were grown in a greenhouse with a temperature range of approximately 18 (night) to 32°C (day) and natural light until used for inoculation studies. They were watered as needed, but were not fertilized.

Corresponding author: B. R. Kropp; E-mail address: brkropp@cc.usu.edu

Publication no. P-1996-0614-02R

Artificial inoculation of plants. Inoculum was prepared from diseased woad plants collected in the field during June in northern Utah. The plants were air-dried at room temperature, and leaves bearing teliosori were subsequently stripped by hand from the plants and crushed into small fragments. The crushed leaf pieces were stored in sealed plastic boxes at –20°C until used to inoculate plants. Before use, the inoculum was surface-disinfested for 1 to 2 min in 10% sodium hypochlorite and rinsed with sterile, distilled water to remove excess sodium hypochlorite. This was necessary to reduce extraneous bacterial and fungal growth that often developed on the inoculum by the end of the inoculation period.

The methods of Morin et al. (13) were modified for experiments on artificial inoculation. One hundred milligrams of the dried leaf inoculum was spread over the surface of a 10-cm-diameter petri dish containing 1.5% water agar, and the leaf pieces were arranged so that the teliosori faced up on the surface of the agar. The petri plates were placed face down over the center of the plant (Fig. 1) and incubated in a dew chamber (Percival Manufacturing Co., Boone, IA). This allowed basidiospores produced by the germinating teliospores to settle onto the leaf surfaces. Dew chamber conditions were maintained between 11 and 17°C for 72 h with a 12-h photoperiod. Dew formation occurred during the dark period. One set of 15 plants and another of 18 plants, both 9- to 10-weeks old, were inoculated over the center of the plant. An additional set of 18 plants was inoculated in the same way except that only one leaf was inoculated, and 20 control plants were not inoculated. The plants were transferred to a greenhouse for 4 to 6 weeks before being moved outside for further growth.

For studies on the early colonization of woad, 30 mg of surface-disinfested inoculum was added to each 60-cm-diameter petri plate containing 1.5% water agar. Each plate was inverted over the apex of a single leaf of a 9- to 10-week-old dyer's woad plant (Fig. 1). The inoculated leaves averaged 6 cm in length (including the petiole), and approximately 1.5 cm of the leaf was exposed to

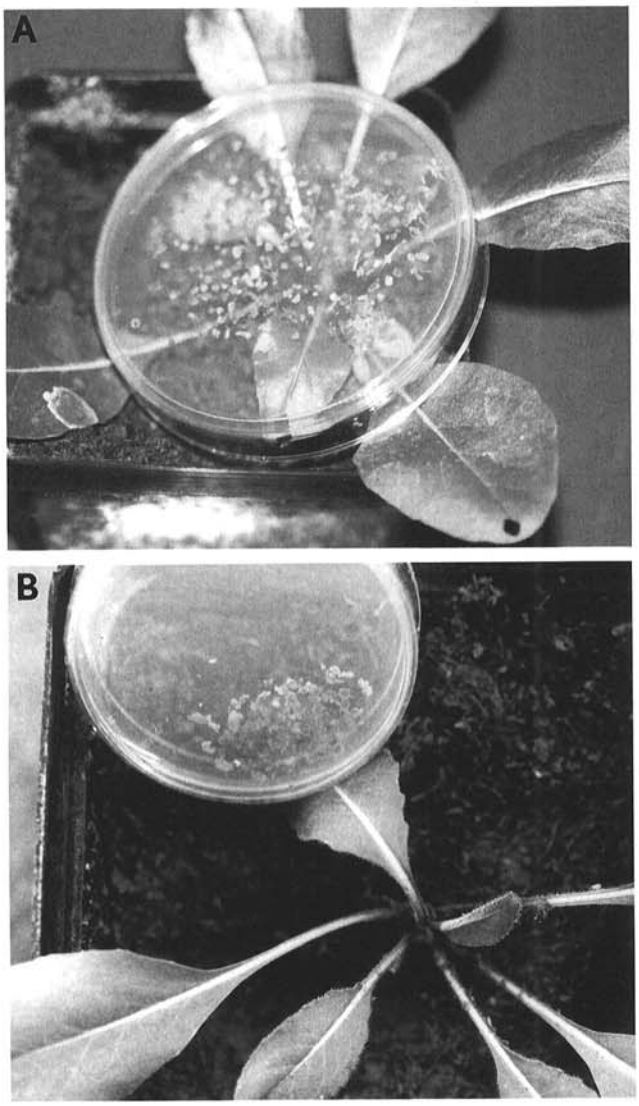

Fig. 1. Method for inoculating **A,** whole plants and **B,** leaf tips of dyer's woad with *P. thlaspeos*. Bits of teliosorus-bearing dyer's woad leaves are suspended on water agar, allowing basidiospores to fall onto the plants.

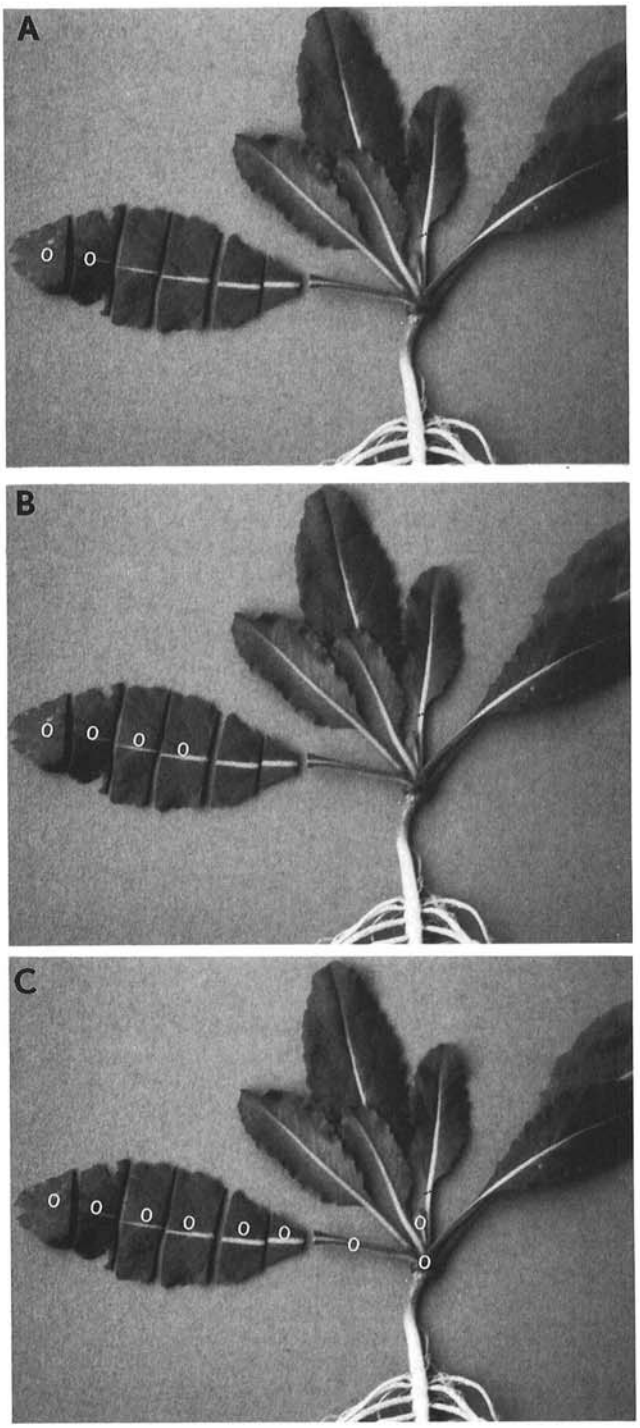

Fig. 2. Representation of the colonization of dyer's woad plants by *Puccinia thlaspeos* after inoculating the tip of a single leaf. Infection was determined over a 10-week period by polymerase chain reaction. **A,** Movement of the fungus through the leaf 1 week after inoculation (circles indicate presence of *P. thlaspeos*). **B,** Movement of *P. thlaspeos* 5 weeks after inoculation. **C,** Movement of *P. thlaspeos* 10 weeks after inoculation.

the inoculum. Care was taken to place the petri plate face down over the leaf to minimize exposure to the rest of the plant, and each inoculated leaf was marked by clipping it slightly at the tip. Twelve plants were inoculated in this fashion. The plants were grown under greenhouse conditions until the end of the study, and the experiment was repeated twice.

To follow the colonization process, three replicate plants were harvested from each experiment at 1, 3, 5, and 10 weeks after inoculation. The leaves that had been inoculated were removed, thoroughly washed in tap water, cut into segments 0.5 to 1.0 cm in length (Fig. 2), and air-dried at room temperature. Each leaf was traced, and the position of each segment was noted to document rust movement through the leaf using polymerase chain reaction (PCR).

DNA extraction and amplification. Because infected dyer's woad is often asymptomatic during the first growing season, the movement of the rust through the leaves was followed using PCR to detect rust in successive leaf segments. DNA was extracted from the dried leaf samples using the method of Kropp et al. (10). Between 0.025 and 0.040 g of dried leaf tissue was crushed to powder in liquid nitrogen inside a 1.5-ml microcentrifuge tube using a glass rod. Five hundred microliters of extraction buffer (0.7 M NaCl, 0.05 M Tris-HCl (pH 8), 0.01 M EDTA, 1% 2-mercaptoethanol, and 1% cetyltrimethylammonium bromide [CTAB]) was added to each crushed sample. After incubation at 60°C for 30 min, an equal or greater volume of chloroform/isoamyl alcohol (24:1 by volume) was added to each tube. The tubes were vortexed to emulsify the mixture and centrifuged at 12,000 rpm (11,750 × *g*) for 5 min. Nucleic acids were precipitated from the resulting supernatant using an equal or greater volume of cold (–20°C) isopropyl alcohol and collected by centrifugation at 12,000 rpm (11,750 × *g*) for approximately 5 min. Each pellet was washed in 80% ethyl alcohol, recentrifuged, and allowed to dry overnight. The nucleic acids were resuspended in 20 to 40 µl of Tris-EDTA (TE-8) before use. For PCR, the DNA solution was usually diluted 1:10 (by volume) with TE-8; but dilutions of 1:100 or 1:1,000 were also used when necessary to achieve amplification.

Two pairs of primers were used for PCR amplification of part of the 5′ end of the large ribosomal subunit DNA (10). Primers F63 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and R635 (5′-GGGTCCGTGTTTCAAGACGG-3′) correspond to highly conserved regions of the subunit (8,9) and were used to ensure that amplifiable DNA had been extracted from each sample. A second primer set consisting of F63 along with a rust specific primer, Rust1 (5′-GCTTACTGCCTTCCTCAATC-3′), was used to detect the rust (11).

Each 25-µl PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.2 mM each of dNTPs, 0.1 µM 5′ primer, 0.1 µM 3′ primer, and 0.6 units *Taq* DNA polymerase. One microliter of diluted DNA extract was used per reaction. Reactions were covered with one drop of mineral oil to prevent evaporation and were carried out using a Perkin-Elmer DNA thermocycler (Perkin-Elmer Corp., Norwalk, CT). The following amplification parameters were used: denaturation at 94°C for 30 s, annealing at 55°C for 1 min, and extension at 72°C for 2 min. After 40 cycles, the reaction was held at 72°C for 10 min and terminated.

Colonization of mature plants. Naturally infected dyer's woad plants in their second season were collected in the field at three developmental stages: the plants were either i) still bolting, but had not produced flowers; ii) in the process of flowering; or iii) had produced seed. The selected plants were all diseased, and each had produced at least one asymptomatic shoot from the rosette. They were removed from the soil along with part of their root system, pressed, and air-dried. One plant at each developmental stage was collected. An asymptomatic plant in the rosette stage was also collected in late autumn. This plant had been grown in a pot as described above and was inoculated by being exposed to infected plants in the field before being transplanted to a nursery. The rosette was asymptomatic but, prior to collection, it had been shown to be rust-infected using the PCR-based method of Kropp et al. (10).

Samples were taken every few centimeters from the dried leaves and stems of both the symptomatic and asymptomatic shoots of each of the above plants. Samples were also taken from the roots of each plant. The samples were carefully rehydrated and washed in water using a dissecting scope to make sure that extraneous surface material was removed. The silicles of the seed were carefully washed, and the epidermis was stripped from the roots and from some stem samples to further assure removal of surface material. DNA was extracted from each sample, and the presence of rust was determined using PCR as described above.

Seed viability. The germination of seed collected in the field from healthy plants was compared with that of seed from asymptomatic and symptomatic shoots of infected plants. The seed were collected from three plants with both symptomatic and asymptomatic shoots, as well as from three healthy plants. Each of the infected plants was chosen because some of the seed produced on the infected branches bore teliosori. The seed were removed from their silicles and germinated at room temperature on moistened filter paper in a sterile, plastic petri dish. Twenty seed from each healthy plant were compared with 20 seed from the asymptomatic and symptomatic shoots of the diseased plants. Only 10 seed were available from one of the symptomatic shoots, and 40 were used from the asymptomatic shoot of another. These were divided into replicate petri plates containing five seed each. The data for percent germination were analyzed using *t* tests after being pooled and arcsin-transformed.

RESULTS

Artificial inoculation. Dyer's woad was inoculated successfully and reproducibly using crushed leaf pieces from diseased plants suspended on water agar plates above the plants. The inoculations were considered successful when symptoms appeared on the plants. All of the plants in one group and 94% of those in a second group were infected by suspending inoculum over the center of the plant (Table 1). When only one leaf was inoculated, only 28% of the plants became diseased (Table 1). The first symptoms appeared between 10 and 13 weeks after inoculation and continued to appear on previously asymptomatic plants for a period of 9 months. Initial symptoms were the yellowing and distortion of new leaves produced at the center of the rosettes, followed by the appearance of spermatia on the leaves.

Initial events in the colonization of dyer's woad. When plants were inoculated at the distal end of a single leaf, *P. thlaspeos* readily infected the inoculated apices and gradually moved down the leaf and through the petiole where it systemically infected the entire plant. After 1 week, the fungus could be detected by PCR only in those apical leaf segments exposed to inoculum. Although plant DNA was consistently amplified using the conserved primer pair (Fig. 3), there was no amplification using the rust-specific

TABLE 1. Percent artificially inoculated plants showing disease symptoms by 9 months after inoculation

	Percent symptomatic plants
Uninoculated controls	0
Whole-plant inoculation 1[a]	100
Whole-plant inoculation 2	94
Single-leaf inoculation[b]	28

[a] Two groups of plants (one with 18 plants, the other with 15) were tested by covering the center of the rosette, along with most of the leaves, with an inverted petri plate containing inoculum.

[b] One group of 18 plants was tested by inoculation of a single leaf.

primers from the other leaf segments. This was consistent for all six plants tested. On average, by 5 weeks after inoculation, the fungus was detected by PCR in leaf segments from the first 2.75 cm of the leaves (Table 2). Considering that about 1.5 cm of the leaves were exposed to inoculum, this indicates that *P. thlaspeos* moved through the plant tissue at an average rate of 0.25 cm per week.

The rate of colonization varied among the plants. In one case, *P. thlaspeos* moved less than 1 cm down the inoculated leaf after 5 weeks, while in several others, it moved up to 3.5 cm beyond the inoculated portion. By 10 weeks after inoculation, the fungus was detected by PCR in all leaf segments including the petioles of all six tested plants (Table 2, Fig. 2). At 10 weeks after inoculation, *P. thlaspeos* was also present in a new leaf growing from the center of the rosette of one of the plants after inoculation (Figs. 2 and 3).

Distribution of rust in older plants. As expected, *P. thlaspeos* was detected by PCR in all symptomatic parts of naturally infected plants. However, *P. thlaspeos* was also detected in asymptomatic parts of these same plants, regardless of the developmental stage studied (Table 3, Fig. 4). The fall rosette was well-colonized even though it was completely asymptomatic. The rust fungus was present in most of the plant leaves, particularly in leaves at the center of the rosette. Surprisingly, it was also present in the roots of the rosette. Bolted, symptomatic plants were thoroughly colonized by the rust. The fungus was consistently found in the asymptomatic shoots of each of these plants and was present in the stems, leaves, and often in the roots. In one case, rust was detected by PCR in a developing seed produced on an asymptomatic shoot (Table 3).

Effect of rust on seed germination. There was no significant difference in percent germination between seed collected from healthy plants and seed collected from either asymptomatic or symptomatic shoots of infected plants (Table 4). This was true even though, as described above, *P. thlaspeos* was detected in at least one seed from asymptomatic shoots. Since the silicle of the seed had been carefully washed and examined using a dissecting scope to ensure removal of extraneous surface material, it is likely that at least the silicle of the seed can be colonized by the fungus.

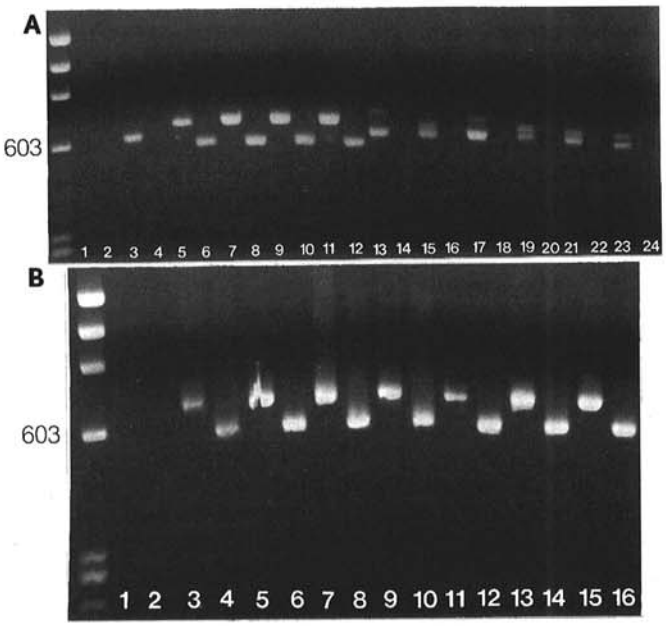

Fig. 3. Agarose gel showing amplification of rust DNA from asymptomatic leaf segments of dyer's woad inoculated with *Puccinia thlaspeos*. **A,** Samples taken 1 week after inoculation of the tip of a single leaf. Lanes 1 and 2, control polymerase chain reaction (PCR) without DNA using conserved primers (F63 and R635) and rust specific primers (R1 and F63), respectively. Lanes 3 and 4, uninfected woad using conserved primers and rust-specific primers, respectively. Lanes 5 and 6, control leaves with rust pustules using conserved and rust-specific primers, respectively. Remaining pairs of lanes show amplification of DNA from segments of the inoculated leaf. The first lane of each pair shows amplification using conserved primers (F63 and R635), the second lane shows amplification using rust-specific primers (R1 and F63). Lanes 7 and 8, 0.5 cm from tip of inoculated leaf; lanes 9 and 10, 1.5 cm from tip of inoculated leaf; lanes 11 and 12, 2.0 cm from tip of inoculated leaf; lanes 13 and 14, 2.5 cm from tip of inoculated leaf; lanes 15 and 16, 3.0 cm from tip of inoculated leaf; lanes 17 and 18, 4.0 cm from tip of inoculated leaf; lanes 19 and 20, 5.0 cm from tip of inoculated leaf; lanes 21 and 22, petiole of inoculated leaf; and lanes 23 and 24, new leaf produced after inoculation. **B,** Samples taken 10 weeks after inoculation of the tip of a single leaf. Lanes 1 and 2, control PCR without DNA using conserved primers (F63 and R635) and rust specific primers (R1 and F63), respectively. Lanes 3 and 4, control leaves with rust pustules using the preceding primers. Remaining pairs of lanes show amplification of DNA from segments of the inoculated leaf. The first lane of each pair shows amplification using conserved primers (F63 and R635), the second lane shows amplification using rust-specific primers (R1 and F63). Lanes 5 and 6, 1.5 cm from tip of inoculated leaf; lanes 7 and 8, 2.5 cm from tip of inoculated leaf; lanes 9 and 10, 3.0 cm from tip of inoculated leaf; lanes 11 and 12, 4.5 cm from tip of inoculated leaf; lanes 13 and 14, petiole of inoculated leaf; and lanes 15 and 16, new leaf produced after inoculation. Marker: ϕX174 replicative-form DNA/*Hae*III digest.

DISCUSSION

Successful artificial inoculation of dyer's woad with *P. thlaspeos* is a key step in studying the biology of this rust. The conditions that we used to carry out the inoculations were comparable with those reported to result in infections by other rusts. Our method was modeled after that of Morin et al. (13) who found that teliospores of *P. xanthii* germinated best in the dark under humid conditions. They also found that *Xanthium occidentale* did not become infected by *P. xanthii* basidiospores in the absence of dew. Our study shows that teliospores of *P. thlaspeos* are able to germinate and infect dyer's woad under similar conditions. Although

TABLE 2. Movement of *Puccinia thlaspeos* through dyer's woad after inoculation of the tip of a single leaf

	Weeks after inoculation[a]			
Average distance from leaf tip	1	3	5	10
1.84 cm	+	+	+	+
2.33 cm	–	+	+	+
2.75 cm	–	–	+	+
4.20 cm	–	–	–	+
Petiole	–	–	–	+
Meristematic area	–	–	–	+
New leaf[b]	–	–	–	+

[a] The presence of *P. thlaspeos* in asymptomatic tissue was detected using polymerase chain reaction (+ = fungus detected, – = fungus not detected). A total of six plants (three from each experiment) was destructively sampled at every time point.

[b] *P. thlaspeos* detected in a new leaf of one of the six plants tested after 10 weeks.

TABLE 3. Presence of *Puccinia thlaspeos*[a] in asymptomatic parts of infected dyer's woad plants

	Stage of plant			
Plant part	Rosette (fall)	Bolting	Flowering	Mature
Root	+	–	+	+
Crown	+	+	+	+
Stem base	np	+	+	+
Mid-stem[b]	np	+	+	+
Stem tip[b]	np	+	+	+
Seed	np	np	+	–

[a] The presence of rust in asymptomatic tissue was detected using polymerase chain reaction (+ = fungus detected, – = fungus not detected, np = plant part not present).

[b] Samples taken from the stem or from the leaves along the stem.

Morin et al. (13) reported an optimal temperature of 20 to 30°C for germination of teliospores of *P. xanthii*, our inoculations were done between 11 and 17°C. We chose lower temperatures for our rust, since teliospores usually appear naturally on dyer's woad during spring and early summer when local night temperatures are relatively cool. Further work is required to determine the optimum conditions for inoculation with dyer's woad rust. The appearance of symptoms over a period of 2 to 9 months after inoculation in artificially inoculated plants is consistent with the pattern seen in nature, in which dyer's woad sometimes shows symptoms at the end of the first summer, but often remains asymptomatic until the spring of the second season (5).

Very little is known about the process by which systemic rusts infect and colonize their hosts. One of the few systemic rusts that has been well-studied is *P. punctiformis*, which is of interest as a potential biocontrol agent for Canada thistle (*Cirsium arvense*). This rust appears to infect its host primarily through the root system. French and Lightfield (6) determined that the best technique for inducing systemic infections of Canada thistle by this rust was to inoculate root cuttings with teliospores. Exposing the leaves of Canada thistle to germinating teliospores did not result in any infections. French et al. (7) also found that an organic compound in crude extracts from Canada thistle roots stimulates teliospore germination.

Nothing has been published on the process of infection and colonization of dyer's woad by *P. thlaspeos*. The lack of symptoms during the first phase of the disease makes the colonization process particularly difficult to study. However, the use of PCR allowed us to follow the movement of the rust during the colonization process in the absence of symptoms. The results provide evidence showing that woad is systemically colonized through leaf infection. After basidiospores infect the leaves, the rust gradually moves through the leaf tissue at a rate of approximately 0.25 cm per week. It then moves into the petiole and can be detected in newly produced leaves after about 10 weeks. The rate at which the fungus moves through the plants appears to vary. In some plants, the fungus had moved much of the way down the inoculated leaf after 5 weeks. In others, it barely moved beyond the inoculated leaf apex. By 10 weeks after inoculation, rust was detected in the meristematic area of two out of six inoculated plants and in a new leaf of one of these plants. Thus, penetration of the rust into leaves of dyer's woad does not always result in colonization of the entire plant. Leaves on rosettes of dyer's woad appear to senesce after about 6 to 10 weeks; unless the fungus moves into the main stem during this period, it would be eliminated from its host. This is consistent with results of our artificial inoculations. Nearly all the plants that were completely exposed to inoculum became systemically infected, while only about a third of those in which a single leaf had been inoculated became systemically infected (Table 1). Leaf senescence probably eliminates many infections in nature before they can systemically invade the plants.

Detection of the rust in the roots and most leaves of an older rosette (Table 3) indicates that it gradually colonizes the entire plant from its point of entry. We propose that, in the field, the fungus infects leaves of the rosette during the summer months of the first year and then moves into the roots where it overwinters. Although the leaves of dyer's woad may survive winter conditions, the migration of the rust into the root system of the plant would increase its chances for survival. The fungus would then be able to colonize new shoots produced by the plant when it bolts the following spring. The systemic colonization also provides a method for the rust to survive range fires. Regrowth from the roots of infected woad plants that had the foliage completely burned off in a range fire in July 1995 was still infected with rust in the spring of 1996 (S. Thomson, *personal communication*).

Diseased plants were also found to be thoroughly invaded by *P. thlaspeos* after bolting. However, the observation that asymptomatic shoots were also well-colonized by the rust was unexpected. We had chosen diseased plants with at least one asymptomatic shoot for this work because of an earlier finding that rust could be detected in asymptomatic leaf samples from bolted plants (10). We originally thought the presence of rust in asymptomatic leaves might have been from new infections during the second season that did not have time to develop symptoms. Although this possibility cannot be completely excluded, the consistent presence of the fungus along the entire length of asymptomatic shoots from plants at several developmental stages indicates that these shoots were infected during the first season.

In most cases, diseased dyer's woad either produces no seed or rarely produces silicles with aborted, shriveled seed inside. As a result, the reproductive potential of dyer's woad is greatly reduced by the rust. However, healthy seed are occasionally produced on both asymptomatic and symptomatic shoots of rusted plants. These seed do not appear to be weakened and germinate as well as seed from uninfected plants. We know of no reports of rusts being seed-borne, but further work needs to be done to determine whether seed produced by systemically infected dyer's woad are able to transmit the disease.

Two additional examples of systemic rusts that affect separate parts of their hosts differently are given by Arthur (1). Branches of the annual plant *Chamaesyce serpyllifolia* that bear different spore stages of the autoecious rust *Uromyces euphorbiae* (= *proëminens*) are morphologically distinct from one another. Those that bear the aecial stage do not flower, are upright, and have long internodes. Yet, branches from the same plant that have the uredial and telial stages are normal in appearance and produce flowers. Another example is *Tranzschelia discolor* (= *punctata*), a heteroecious rust that systemically infects *Hepatica nobilis* var. *acuta* (= *acutiloba*). In his book, Arthur (1) illustrates a plant in which branches infected with the aecial stage of this fungus stand upright, while the normal leaves are prostrate. There is no indication whether the normal leaves of *Hepatica* are colonized by rust, as

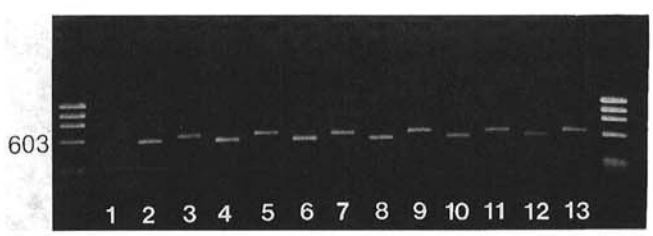

Fig. 4. Agarose gel showing amplification of *Puccinia thlaspeos* DNA from different parts of infected dyer's woad. Lane 1, control PCR reaction without DNA using conserved primers (F63 and R635). Remaining pairs of lanes show amplification of DNA from plant parts, the first lane of each pair shows amplification with rust-specific primers (R1 and F63), the second lane shows amplification using conserved primers (F63 and R635). Lanes 2 and 3, root material; lanes 4 and 5, asymptomatic shoot; lanes 6 and 7, tip of symptomatic shoot; lanes 8 and 9, middle portion of symptomatic shoot; lanes 10 and 11, base of symptomatic shoot; and lanes 12 and 13, control leaves with rust pustules. Marker: ϕX174 replicative-form DNA/*Hae*III digest.

TABLE 4. Percent germination of seed from symptomatic and asymptomatic branches of infected dyer's woad

	% germination[a]
Control[b]	65.0
Asymptomatic branch	60.0
Symptomatic branch	52.0

[a] Germination values were not significantly different ($P = 0.05$) using t tests for comparing control seed with seed from asymptomatic and symptomatic branches and a paired t test to compare germination of seed from the asymptomatic with that from the asymptomatic branches.

[b] Control seed were obtained from healthy plants collected at a site lacking diseased plants.

occurs with dyer's woad. At present, we cannot explain these differential effects other than to speculate that somatic differences between the parts of these plants may alter symptom expression.

The challenge of controlling dyer's woad on much of the rangeland in which it occurs is a difficult one. Because of their effects on native plants and the inaccessibility of woad populations, widespread use of herbicides may be undesirable or impossible. As a result, biological control may be the best strategy. Dyer's woad rust appears to have potential as a biocontrol agent, but, to use it most effectively, we need to better understand its biology. Its host range and taxonomic standing also need further study. Additional studies may be required to determine the optimal conditions for inoculation and to elucidate the factors controlling symptom expression.

LITERATURE CITED

1. Arthur, J. C. 1929. The Plant Rusts. John Wiley & Sons, Inc., New York.
2. Arthur, J. C., and Cummins, G. B. 1962. Manual of the Rusts in the United States and Canada. 2nd ed. Hafner Publishing Co., New York.
3. Callihan, R. H., Dewey, S. A., Patton, J. E., and Thill, D. C. 1984. Distribution, biology and habitat of dyers woad (*Isatis tinctoria* L.) in Idaho. J. Idaho Acad. Sci. 20:18-32.
4. Farah, K. O., Tanaca, A. F., and West, N. F. 1988. Autoecology and population biology of dyers woad (*Isatis tinctoria*). Weed Sci. 36:186-193.
5. Flint, K. M., Thomson, S. V., Dewey, S., and Evans, J. 1993. Timing of infection of dyers woad (*Isatis tinctoria* L.) by a *Puccinia* rust under field conditions. Proc. West. Soc. Weed Sci. 46:116.
6. French, R. C., and Lightfield, A. R. 1990. Induction of systemic aecial infection in Canada thistle (*Cirsium arvense*) by teliospores of *Puccinia punctiformis*. Phytopathology 80:872-877.
7. French, R. C., Turner, S. K., Sonnett, P. E., Pfeffer, P., and Piotrowski, E. 1988. Properties of an extract from Canada thistle roots that stimulates germination of dormant teliospores of Canada thistle rust (*Puccinia punctiformis*). J. Agric. Food Chem. 36:1043-1047.
8. Georgiev, O. I., Nillaev, N., Hadjiolov, A. A., Skryabin, K. G., Zakharyev, V. M., and Bayev, A. A. 1981. The structure of the yeast ribosomal RNA genes. 4. Complete sequence of the 25S rRNA gene from *Saccharomyces cerevisiae*. Nucleic Acid Res. 9:6953-6959.
9. Gueho, E., Kurtzman, C. P., and Peterson, S. W. 1989. Evolutionary affinities of heterobasidiomycetous yeasts estimated from the 18S and 25S ribosomal RNA sequence divergence. Syst. Appl. Microbiol. 13:230-236.
10. Kropp, B. R., Albee, S., Flint, K. M., Zambino, P., Szabo, L., and Thomson, S. V. 1995. Early detection of systemic rust infections of dyers woad (*Isatis tinctoria*) using the polymerase chain reaction. Weed Sci. 43:467-472.
11. Liu, Z., Szabo, L. J., and Bushnell, W. R. 1993. Molecular cloning and analysis of abundant and stage-specific mRNAs from *Puccinia graminis*. Mol. Plant-Microbe Interact. 6:84-91.
12. Lovic, B. R., Dewey, S. A., Thomson, S. V., and Evans, J. O. 1988. *Puccinia thlaspeos*—A possible biocontrol agent for dyers woad. Proc. West. Soc. Weed Sci. 41:55-57.
13. Morin, L., Brown, J. F., and Auld, B. A. 1992. Effects of environmental factors on teliospore germination, basidiospore formation, and infection of *Xanthium occidentale* by *Puccinia xanthii*. Phytopathology 82:1443-1447.
14. Varga, W. A., and Evans, J. O. 1978. Dyer's woad: From cultivated to cursed. Utah Sci. 39:87-89.
15. Young, J. A., and Evans, R. A. 1971. Germination of dyers woad. Weed Sci. 19:76-78.

Your Company is Invited to Join These Leaders...
APS Sustaining Associate Members

ABBOTT LABORATORIES, Long Grove, IL
AGDIA, INC., Elkhart, IN
AGREVO USA COMPANY, Wilmington, DE
AGRICULTURE & AGRI-FOOD CANADA, Sidney, British Columbia, Canada
AGRICULTURE & AGRI-FOOD CANADA, Vineland Station, Ontario, Canada
ALF CHRISTIANSON SEED CO., Mt. Vernon, WA
AMERICAN CYANAMID COMPANY, Princeton, NJ
BALL HORTICULTURAL COMPANY, West Chicago, IL
BASF CORPORATION, Research Triangle Park, NC
BAYER CORPORATION, Kansas City, MO
BIOLOG, INC., Hayward, CA
BOTANIC GARDENS OF ADELAIDE, Adelaide, Australia
BUCKMAN LABORATORIES, Memphis, TN
BUSCH AGRICULTURAL RESOURCES, INC., Ft. Collins, CO
CEREAL RESEARCH INSTITUTE, Szeged, Hungary
CIBA CROP PROTECTION, Greensboro, NC
DEKALB GENETICS CORP., DeKalb, IL
DEL MONTE FOODS, Gilroy, CA
DOW ELANCO, Indianapolis, IN
DUPONT COMPANY, Wilmington, DE
ELF-ATOCHEM NORTH AMERICA, Philadelphia, PA
GRIFFIN CORP., Valdosta, GA
GUSTAFSON, INC., Plano, TX
HEINZ USA, Stockton, CA
INSTITUTE FOR FOREST & LANDS, Birmensdorf, Switzerland
INSTITUTE OF ARABLE CROPS RESEARCH, Hertfordshire, England
ISK BIOSCIENCES CORPORATION, Mentor, OH
JANSSEN PHARMACEUTICA, Titusville, NJ
K & N EFTHYMIADIS S. A., Athens, Greece
LANDIS INTERNATIONAL, INC., Valdosta, GA
MAHARASHTRA HYBRID SEEDS COMPANY, Bombay, India
MERCK & CO., INC., Rahway, NJ
MONSANTO COMPANY, St. Louis, MO
NORTHRUP KING CO., Stanton, MN
ORSETTI SEED COMPANY INC., Hollister, CA
PERKIN-ELMER/APPLIED BIOSYSTEMS, Foster City, CA
PEST PROS, INC., Plainfield, WI
PIONEER HI-BRED INTERNATIONAL, INC., Johnston, IA
PRIMARY INDUSTRIES - S.A., Loxton, Australia
RHONE-POULENC AG COMPANY, Research Triangle Park, NC
RICERCA, INC., Painesville, OH
ROGERS SEED CO., Nampa, ID
ROHM & HAAS COMPANY, Philadelphia, PA
SAKATA SEED AMERICA, INC., Salinas, CA
SANDOZ AGRO, INC., Des Plaines, IL
THE SCOTTS COMPANY, Marysville, OH
SEMINIS VEGETABLE SEEDS, San Juan Bautista, CA
S & G SEEDS B.V., Enkhuizen, the Netherlands
SOUTHCORP WINES, Nuriootpa, Australia
STA LABORATORIES, INC., Longmont, CO
THE TRYON GROUP, Madison, WI
UNIROYAL CHEMICAL COMPANY, Bethany, CT
UNIVERSITEITSBIBLIOTHEEK, Amsterdam, the Netherlands
VALENT USA CORPORATION, Walnut Creek, CA
WILBUR-ELLIS COMPANY, West Burlington, IA
ZENECA AG PRODUCTS, Richmond, CA

Help Support the Field as Well as Continued Research in Plant Science

How Membership Benefits Your Organization:

PUBLIC RECOGNITION
- Monthly listing in ***Phytopathology***
- Listing in ***Annual Membership Directory***-Salute to Sustaining Associates
- Listing in ***Annual Meeting Program***: badge ribbons designate membership

INFORMATION SERVICES
- **FREE with membership—two of these three journals: *Plant Disease, Phytopathology*, or *Molecular Plant-Microbe Interactions*®**
- ***Phytopathology News***, the Society's newsletter
- Bulletin Board Service, your computer link to APS

TECHNICAL EXPERTISE
- APS Annual Meeting—workshops, symposia for international cooperation in plant science research

JOB PLACEMENT SERVICE
- APS helps you recruit plant science professionals

SAVINGS
- Discounts on APS PRESS books and APS Annual Meeting fee
- Choose one of these (call for information)
  - Save 50% on full page ad in ***Plant Disease***, ***Molecular Plant-Microbe Interactions***, ***Phytopathology News***, ***Annual Membership Directory***, or ***Annual Meeting Program***
  - One customized APS membership list

APS Sustaining Associate members contribute their knowledge, expertise and professional involvement to ensure the continued strength of APS and to promote excellence in plant science. For more information about these organizations, see the *APS Membership Directory*. (Address, telephone, and fax numbers are provided.) Or, you may call APS Headquarters. **If you would like to become a Sustaining Associate member**, call APS Member Services at 1-800-328-7560.