Factors Influencing Rust (*Melampsora apocyni*) Intensity on Cultivated and Wild *Apocynum venetum* in Altay Prefecture, China

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ABSTRACT

Rust (*Melampsora apocyni*) on *Apocynum venetum* is the major constraint to the commercial development of this medicinal herb. To determine the factors influencing rust intensity (maximum disease index [DI_{max}]), rust was investigated from 2011 to 2015 in both cultivated and wild *A. venetum* plants. Partial least squares path modeling (PLS-PM) was used to analyze the paths and extent of the factors related to pathogen, environment, and host that affect rust intensity. DI_{max} exhibited considerable variations across years and study sites, with variations linked to various factors fostering disease development. PLS-PM explained 80.0 and 70.1% of variations in DI_{max} in cultivated and wild plants, respectively. Precipitation was the key factor determining DI_{max} in both cultivated and wild plants (path coefficient [PC] = 0.313 and 0.544, respectively). In addition, the topsoil water content in cultivated plants and the total vegetation coverage in wild plants were also critical determinants of

DI_{max} via their effects on the microclimatic factor (contribution coefficients [CC] = 0.681 and 0.989, respectively; PC = 0.831 and 0.231, respectively). In both cultivated and wild plants, host factors were mainly dominated by *A. venetum* density (CC = 0.989 and 0.894, respectively), and their effect on DI_{max} via the microclimatic factor (PC = 0.841 and 0.862, respectively) exceeded that via the inoculum factor (PC = 0.705 and 0.130, respectively). However, the indirect effects led to DI_{max} variation, while the dilution effect on host (CC = 0.154) from weed in wild plants led to the indirect effect size in wild plants of 0.200, which was lower than −0.699 in cultivated plants.

Keywords: *Apocynum venetum*, cultivated crop, disease index, path modeling, wild plant

Apocynum venetum belongs to the genus *Apocynum* of the family Apocynaceae and is widely distributed throughout Europe and in west, central, and east Asia. It is a perennial herbaceous shrub with a height of 0.8 to 3 m (Gao et al. 2015). During winter, the aboveground parts of the plant die but regenerate in spring, with new stems arising from spreading rhizomes (Thevs et al. 2012). Leaves of *A. venetum* are used as a traditional Chinese medicine and have been promoted as a nutritional tea supplement that has become increasingly popular in both North American and East Asian health food markets (Song and Zhou 2015). China remains the largest producer of *A. venetum* for human use, using leaves obtained mainly from the harvesting of wild plants. However, because there remain only two wild populations of this plant species in Altay Prefecture (Gao et al. 2015), more than 2,000 ha of cultivated *A. venetum* fields have been established since 2009 in Altay Prefecture, China. This is the only commercial cultivation of *A. venetum* worldwide and achieves an annual estimated value of tea products of U.S. \$13.6 million.

Rust, caused by *Melampsora apocyni*, which belongs to the genus *Melampsora* of the family Melampsoraceae, is the major disease affecting *A. venetum* in several Asian and European countries (Gao et al. 2017). It was first observed on wild *A. venetum* in Russia (Tranzschel 1891) and has subsequently been reported on wild or cultivated *A. venetum* in Turkmenistan (Saccardo 1895), Japan

(Hiratsuka 1937), Kazakhstan (Nevodovskii 1956), Bulgaria (Denchev 1995), and Turkey (Kirbağ 2004). In China, since *M. apocyni* was first reported on wild *A. venetum* in Hebei Province in 1951 (Tai 1979), its distribution has expanded to wild or cultivated *A. venetum* in three other provinces or autonomous regions of China, including Henan (Tai 1979), Shanxi (Hu and Qian 1985), and Xinjiang (Zhuang 1989). In Altay Prefecture of Xinjiang, rust symptoms were first observed in 2009 on the leaves of both wild and cultivated *A. venetum*. The disease severely damages leaves, making them unsuitable for tea production and resulting in large economic losses (Gao et al. 2017). This necessitated studies into the factors influencing the intensity of rust occurrence, including the interactions of the pathogen with its host and the environment as a precursor to developing effective control procedures (Zadoks and Schein 1979).

Pathogenicity and host resistance are important factors influencing plant disease development (McCracken et al. 2000). Previous studies on rust caused by the genus *Melampsora* confirmed that, specifically, the presence of urediniospores leads to repeated cycles of infection during the growing season (Guo et al. 2010; Widin and Schipper 1980). Gao et al. (2014) showed that there were no differences in pathogenicity of *M. apocyni* urediniospores from cultivated versus wild plants of *A. venetum* in Altay Prefecture across 2012 to 2015. Both Peng et al. (2008) and Gao et al. (2015) confirmed that wild *A. venetum* plants in Altay Prefecture had a genetic relationship and morphological characteristics similar to those of cultivated plants. This is likely because cultivated plants had been propagated from seed that were collected from wild plants. However, variations in the *A. venetum* density in both cultivated and wild plants, and in their numbers of urediniospores that are spread by the wind, results in variable host–pathogen encounter rates and, consequently, in varying rust intensity in both cultivated and wild plants (Cox et al. 2004).

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For the genus *Melampsora*, urediniospore germination requires near 100% relative humidity or the presence of free moisture on the leaf surface, and occurs across temperatures from 5 to 28°C (Toome et al. 2010; Vialle et al. 2011). Urediniospores typically germinate and form two germ tubes, usually within 6 to 8 h. One of these germ tubes infects the host leaf via stomata, generally within 24 h (Guo et al. 2010). However, in Altay Prefecture, a relative air humidity of near 100% is rare from May to September because this region has a continental semiarid climate (Habiken and Zhao 2011). Despite this, a large volume of rainwater is intercepted by the canopy of plants and adheres to the surface of foliage as droplets or water films (Huber and Gillespie 1992). Weather conditions, particularly both precipitation and temperature, are likely the key determinants of *A. venetum* rust intensity in Altay Prefecture. The microclimate within canopies of plants is also an important factor influencing rust development (Waggoner 1965). Furthermore, heat and water exchange continuously occur through air within and outside plant canopies and from the soil surface by way of topsoil water evaporation and from air movement. In this way, the topsoil water content and canopy density or vegetation coverage directly affect the microclimate in agricultural systems (Norman 1982). Changes in microclimate may also differ at various heights above the ground. As such, these changes lead to differences in timing and intensity of disease occurrence on different parts of plant (Costes et al. 2013; Schmitz and Grant 2009). Recognition of the vertical spread and development characteristics of rust on *A. venetum* would greatly contribute to our understanding of the relationship between the rust development process and the environmental factors encountered by the crop.

Although three alternate hosts for *M. apocyni* have been found—*A. sibiricum* in Kazakhstan (Nevodovskii 1956) and *Pocynum pictum* and *P. hendersonii* in China (Gao et al. 2015; Xu et al. 2013)—no such alternate hosts of *M. apocyni* occur in Altay Prefecture (Gao et al. 2017). In Altay Prefecture, weeds in *A. venetum* fields cannot be removed by either mechanical or other methods because of the requirement that all plants (including weeds) of the local ecological environment should be preserved (Ping et al. 2014). However, the weeds that are present could increase the closure of plant canopies, thus increasing the risk of rust epidemics (van Bruggen and Finckh 2016). In contrast, the presence of weeds that are not susceptible to rust could decrease host–pathogen encounter rates, resulting in decreased disease intensity (Mitchell et al. 2002). Furthermore, weeds in cultivated fields and among wild populations of *A. venetum* may affect the frequency at which *M. apocyni* urediniospores encounter *A. venetum* or alter the microclimate, thus leading to changes in rust intensity. For these reasons, the role of weeds in influencing rust intensity in cultivated and wild *A. venetum* plants needs careful consideration.

The specific objectives of this research were to (i) characterize the variation in rust intensity of both cultivated and wild plants across years, plant vertical direction, and sample sites during a 5-year period of rust occurrence; (ii) determine the path and extent of the factors related to pathogen, environment, and host that affect rust intensity using partial least squares path modeling (PLS-PM); and (iii) establish the critical determinants of rust intensity in both cultivated and wild plants.

MATERIALS AND METHODS

Locations. Research was conducted at two locations (approximately 5 km apart) near the Alakak Township in Altay Prefecture of the Xinjiang Uyghur Autonomous Region, China. One was close to Lake Honggou, and contained a wild population of *A. venetum* (Honggou, 47°39'N, 87°31'E, altitude 483 to 485 m, area 1.47 ha). The second was situated near Lake Alakak and was also a wild population of *A. venetum* (Alakak, 47°42'N, 87°33'E, altitude 492 to 547 m, area 5.23 ha). Nearby, two cultivated fields of *A. venetum* had been established in 2010 (CV2010, 47°44'N, 87°32'E, altitude 510 m, area 133 ha) and 2011 (CV2011, 47°43'N, 87°32'E, altitude

482 to 516 m, area 866 ha) using plants grown from seed collected from local wild *A. venetum*.

In wild plants, the emergence of *A. venetum* stems began during early June. The soil was saline-alkali pH 7.8 to 8.2. From 2011 to 2015, no attempts were made to control rust affecting wild *A. venetum*. In cultivated plants, soil was a sandy loam with a pH of 7.2 to 7.5, and *A. venetum* rows were spaced 1 and 3 m apart in CV2010 and CV2011, respectively. All of these cultivated *A. venetum* plants were spaced 1 m apart within rows. Emergence of *A. venetum* stems began in the middle of April and *A. venetum* were irrigated via drip irrigation every 2 days in 2011 and on 6 or 8 days per year from 2012 to 2015. In 2011 to 2013, no disease control measures were applied to cultivated *A. venetum*, with the exception of the fungicide triadimefon sprayed onto leaves by the grower twice per month in June and August of 2014 and 2015 to control rust.

Experimental design and sampling methodology. In CV2010, CV2011, Honggou, and Alakak, four repeated sampling plots each were chosen (each 20 by 30 m²) that were separated from other plots by an interval of 20 to 25 m, arranged at four different directions (east, west, north, and south), pegged, and used to monitor rust from 2011 to 2015. Within each repeated sampling plot, five quadrats (5 × 5 m²) were pegged and the variables data in relation to topsoil water content, vegetation, and *A. venetum* plant in each quadrat were collected. Furthermore, the mean value of five quadrats of each plot was recorded as one repeated sampling value of each site. However, in 2014 and 2015 due to the fungicide (triadimefon) spraying onto leaves to control rust in cultivated *A. venetum*, rust was not monitored and data for these variables were not collected. Overall, there were two cultivated plants × wild plants = four treatments with 16 plots.

Weather data collection. Air temperatures (°C) and precipitation (in millimeters) were recorded every 15 min from May to September of each year via an automatic meteorological observation station (EM50; Decagon Devices, Inc., Pullman, WA) located at CV2011. Daily average temperatures and daily accumulated precipitation were calculated from daily records.

Estimation of urediniospore concentration in the air. Using a microbiological air sampler (Surface Air System Super 100; pbi Company, Milan, Italy), the concentration of *M. apocyni* urediniospore in the air in both cultivated and wild plants was measured every 2 days during the growing seasons from 2012 to 2015. One sampler was placed at the middle of each plot of CV2010, CV2011, Honggou, and Alakak. The sampler intake was at a height of 0.5 m, with air sampled daily from 9.00 A.M. local time until 9.00 A.M. the following day. For daily sampling, the samplers were set to suck air at 30-min intervals until a total of 48 liters of airflow had passed through a detachable head with holes and had been directed onto the Vaseline-covered surface of a 9-cm-diameter Petri dish that was tightly inset in the head. Urediniospores adhering to the Vaseline-covered surface were identified using light microscopy according to their particular morphological characteristics, and the numbers of urediniospores present were recorded. The daily urediniospore concentration was expressed as the number of spores per cubic meter of air. The accumulated urediniospore concentration (AUC), before the disease index (DI) reached maximum value, was calculated for each year as follows:

$$AUC = \sum_{i=1}^N [(y_i + y_{i+1})/2][t_{i+1} - t_i] \quad (1)$$

where y_i is the urediniospore concentration at the i^{th} observation, N is the number of observations, and t_i is the duration (in days) of the i^{th} observation.

Topsoil water, vegetation, and *A. venetum* plant characteristics. In early June, July, and August of each year, topsoil was sampled to a depth of 20 cm using a soil auger at the central position of a single bush at the middle of each quadrat. Soil samples were immediately sealed in an aluminum box. The fresh weight of the soil was measured in the laboratory, then oven dried at 105°C for 48 h to determine the

dry weight. The soil water content was presented as a percentage of the dried soil weight. The upper limit of the soil water content per quadrat was determined according to the gravimetric method developed by Bletsos et al. (1999). The relative soil water content is presented as a percentage of the upper limit of soil water content.

During the same period, the number of *A. venetum* stems in each quadrat was counted, and the total vegetation coverage and species diversity in each quadrat was measured using the point intercept method developed by Goodall (1952). The total vegetation coverage was estimated by recording the total number of vertical survey pin contacts with foliage. The coverage of each species was

estimated by recording the number of species touched by each pin. The coverage data were expressed as the percentage of ground covered by all species or each species. A steel tape was used to measure the vertical height (in centimeters) of plants in each quadrat. The Shannon-Wiener index of diversity (H') for the community of each plot was computed with the following formula:

$$H' = -\sum (P_i \ln P_i) \quad (2)$$

where $P_i = (h_i/H + c_i/C)/2$; H and C are the cumulative height and cumulative coverage, respectively, of all species in the quadrat

Fig. 1. Daily precipitation (mm) and temperature (°C) from mid-May to mid-September for 2011 through 2015 in Altay Prefecture of the Xinjiang Uygur Autonomous Region, China.

population; and h_i and c_i are the mean values of height and degree of cover, respectively, belonging to the i th species (Yang et al. 2011). Mean values of each variable in relation to topsoil water, vegetation, and *A. venetum* plants across the months from the date when rust was initially observed to the date DI reached maximum value were calculated for each year.

Monitoring of rust. Each year, the disease was evaluated at CV2010, CV2011, Honggou, and Alakak every 7 to 10 days from early May until the aboveground plant parts died (approximately at the end of August to early September). Ten *A. venetum* shrubs of roughly similar size and height were randomly chosen and marked in each plot; five stems of each shrub, including one at the center, were marked for rust assessment. Three leaves were randomly selected at each of the three sampling heights—10 cm (lowest), 11 to 50 cm (middle), and >50 cm (upper) above the ground of each marked stem—and rust severity was assessed on these. From each plot, in total, 450 leaves were assessed nondestructively for each assessment. Rust severity was estimated visually using an ordinal scale as the percentage of observed leaves covered by uredinia, as follows: 0 = no signs of rust infection and 1 = 0.1 to 5, 2 = 5.1 to 25, 3 = 25.1 to 50, 4 = 50.1 to 75, and 5 = 75.1 to 100% of leaf area covered with uredinia. The disease assessments ceased when aboveground plant parts died. DI was computed as a comprehensive index that included both disease severity and disease incidence (Harveson and Rush 2002; Ma et al. 2001), as follows:

$$DI = \left[100 \times \sum_i (i \times L_n) \right] / (5 \times LN) \tag{3}$$

where i is the disease severity scale ($i = 0, 1, 2, 3, 4$, and 5) and LN and L_n are the total number of leaves and the number of leaves of each disease severity, respectively (Zhang et al. 2007). DI at each height was calculated and used to analyze the disease development on different plant parts. The mean DI of the three heights of each assessment was used to draw the temporal disease development curve. Following this, the maximum DI (DI_{max}) in each disease development curve was excerpted as rust intensity.

Analysis of variables related to rust intensity. PLS-PM is a data analysis approach for studying observed variables that can be summarized by a latent variable (i.e., a variable that cannot be measured directly) and whether linear relationships exist between latent variables. The advantages of PLS-PM include the ability to

handle multicollinearity among independent variables, robustness in the face of data noise or missing data, and the fact that it allows the creation of independent latent variables directly on the basis of cross-products involving the response variables, leading to stronger predictions (Sanchez 2013). Therefore, because the data of this study showed multicollinearity, missing entries, and a small sample size ($n < 100$), PLS-PM was the most appropriate means to analyze interactions between rust density and its influencing factors.

First, we constructed the path model based on theoretical factors, knowledge, and logical assumptions about rust intensity (Lundquist 2007). We assumed that the weather and microclimatic within canopies of plants, host, and inoculum (amount of captured *M. apocyni* urediniospores) factors were the latent variables affecting rust intensity in both cultivated and wild plants. The formative relationships between these latent variables and the measurement variables (also known as manifest variables in PLS-PM) were defined as follows: the weather factor was dominated by the daily average air temperatures and cumulative precipitation, the microclimatic factor was dominated by the topsoil water content and the total vegetation coverage, the host factor was dominated by *A. venetum* density and species diversity, and the inoculum factor was dominated by the accumulated urediniospore concentration in the air. We assumed that (i) the weather, microclimatic, and inoculum factors directly affected rust intensity (DI_{max}); (ii) the weather factor indirectly affected rust intensity via microclimatic or host factors; and (iii) the host factor had no direct effect on rust intensity but indirectly affected rust intensity via microclimatic or inoculum factors. The relationships between factors were connected by arrows and a path modeling diagram was constructed. Second, groups of data for analysis in cultivated ($N = 24$) and wild ($N = 40$) plants were created to a file of jobsat.csv and imported into the SmartPLS program (version 3.0; SmartPLS GmbH, Boenningstedt, Germany). Path modeling diagrams of both cultivated and wild plants were drawn in the Motivation Module of this program. Finally, the PLS-algorithm procedure of this program was applied to evaluate the validity of the models using the goodness-of-fit (GOF) statistic, which is a measure of their overall predictive power. If $GOF > 0.7$, the models were assessed to be valid, and then the procedure was continued to estimate the contribution coefficients (CC) of manifest variables to the latent variables, the standardized path coefficients (PC, representing the direction and strength of the linear relationships between latent variables), and the explained variability values (R^2) associated with response variables (Kou et al. 2017). In addition, direct effects were obtained via PC for every connecting arrow between latent variables and indirect effects, calculated as the sum of multiplying these values (Sanchez 2013). The bootstrap-algorithm ($N = 1,000$) procedure in the SmartPLS program was applied to estimate the mean indirect effect size, its standard error, and the lower and upper critical value of 95% confidence interval (95%CI). This method defined that the indirect effect had statistical significance if the 95%CI excludes zero value (Efron and Tibshirani 1993).

Data analysis. The DI at the three heights and the mean DI at the three heights (X) in each repeated sampling plot were arcsine square-root transformed ($X + 0.5$), while the topsoil water content in each repeated sampling plot were Box-Cox transformed. The aim of these data transformations was to normalize data prior to analysis of variance (ANOVA). Two-way ANOVA was conducted to determine whether there were significant differences in the mean value of four repetitions of the relative soil water content, total vegetation coverage, diversity index, and *A. venetum* density in CV2010, CV2011, Alakak, and Honggou across both month and year. Using one-way ANOVA and t tests, it was determined whether significant differences for the mean value of four repetitions of relative soil water content, total vegetation coverage, diversity index, and *A. venetum* density existed among the four study sites from 2011 to 2013 and between the two wild sites in 2014 and 2015. One-way ANOVA was also used to analyze the variation of DI_{max} among all

TABLE 1. Factorial analysis of variance for the relative soil water content, total vegetation coverage, diversity index, and *Apocynum venetum* density in cultivated and wild *A. venetum* plants^a

Type	Variable	Sources of variation			
		Month (M)	Year (Y)	M × Y	
Cultivated	CV2010	Relative soil water content	NS	**	NS
		Total vegetation coverage	NS	*	NS
		Diversity index	NS	*	NS
		<i>A. venetum</i> density	NS	*	NS
	CV2011	Relative soil water content	NS	**	NS
		Total vegetation coverage	NS	*	NS
		Diversity index	NS	*	NS
		<i>A. venetum</i> density	NS	*	NS
Wild	Alakak	Relative soil water content	*	NS	NS
		Total vegetation coverage	*	*	*
		Diversity index	NS	*	NS
		<i>A. venetum</i> density	*	**	NS
	Honggou	Relative soil water content	*	NS	NS
		Total vegetation coverage	*	*	*
		Diversity index	NS	*	NS
		<i>A. venetum</i> density	*	*	NS

^a NS = not significant and * and ** indicate significant at the 0.05 and 0.01 probability levels, respectively.

sites and years; DI among the three parts of plants for each assessment in each site in each year; and the mean values of relative soil water content, total vegetation coverage, diversity index, and *A. venetum* density across the months from the date when rust was initially observed to the date when DI reached maximum value among all sites and years. When a significant overall *F* test was found via ANOVA, treatment means were compared using Fisher's least significant difference at *P* = 0.05. Finally, the transformed disease indices and topsoil water contents were back transformed to the original scale and regressed as the observed

values. Spearman's rank correlation tests were used to assess the magnitude of the relationships between DI_{max} and the factors influencing rust intensity for both cultivated and wild *A. venetum* (Turechek 2004). These statistical analyses were conducted using the procedures of SPSS (version 19.0; IBM, Armonk, NY, U.S.A.).

RESULTS

Precipitation and temperature. In 2011 and 2013, 25 and 33 rainfall events, respectively, occurred from mid-May to

Fig. 2. A, Relative soil water content; B, total vegetation coverage; C, diversity index; and D, density of *Apocynum venetum* in CV2010 (cultivated *A. venetum* established in 2010), CV2011 (cultivated *A. venetum* established in 2011), Alakak, and Honggou (two wild population of *A. venetum*) in June, July, and August from 2011 to 2015. Vertical lines indicate least significant difference, where *P* < 0.05 and an asterisk (*) indicates a significant difference between Alakak and Honggou at *P* = 0.05 using a *t* test.

TABLE 2. Characteristics of topsoil water, vegetation, and host in cultivated and wild *Apocynum venetum* plants in the period of rust (*Melampsora apocyni*) increase from 2011 to 2015^a

Year	Type		Relative soil water content (%)	Total vegetation coverage (%)	Species diversity	<i>A. venetum</i> density (stems/m ²)	Accumulated urediniospore concentration (spore/m ³)
2011	Cultivated	CV2010	77.5	53	0.6390	34	...
		CV2011	69.2	40	0.5645	18	...
	Wild	Alakak	43.7	75	0.7329	22	...
		Honggou	61.1	88	0.6802	37	...
2012	Cultivated	CV2010	65.1	58	0.6506	39	93
		CV2011	57.2	45	0.7390	21	76
	Wild	Alakak	38.7	71	0.7978	19	60
		Honggou	56.7	81	0.7627	31	58
2013	Cultivated	CV2010	81.1	61	0.7540	37	96
		CV2011	74.1	46	0.7218	22	82
	Wild	Alakak	42.7	70	0.8271	18	71
		Honggou	61.0	83	0.8010	25	61
2014	Wild	Alakak	45.2	64	0.8041	11	33
		Honggou	56.4	79	0.7843	24	31
2015	Wild	Alakak	40.4	60	0.8156	9	35
		Honggou	53.8	76	0.7947	23	5
	LSD ^b	...	7.4	9.2	0.0295	4.1	...

^a Data are the mean value of each parameter in the period that was from the date disease was initially observed to the date disease index reached peak value in each year.

^b Least significant difference at $P < 0.05$.

Fig. 3. Urediniospore concentration of *Melampsora apocyni* in the air in CV2010 (cultivated *Apocynum venetum* established in 2010), CV2011 (cultivated *A. venetum* established in 2011), Alakak, and Honggou (two wild population of *A. venetum*) in the growing seasons from 2012 to 2015.

mid-September, of which 2 and 6 were heavy rain events (precipitation exceeding 8 mm/hour) that occurred in mid- to late June and late May to mid-June, respectively (Fig. 1). The total precipitation was 76.1 and 140.4 mm, respectively. In 2014 and 2015, there were 13 and 22 rainfall events, respectively, that occurred

from mid-May to mid-September. The total precipitation (33.6 and 33.7 mm, respectively) was lower than that observed for 2011 and 2013 (Fig. 1). In 2012, precipitation in May and June was infrequent but both frequency and amount of rain increased in July and August (Fig. 1). In all, 29 rainfall events were observed with a

Fig. 4. Disease index (DI) of rust (*Melampsora apocyni*) over time in CV2010 (cultivated *Apocynum venetum* established in 2010), CV2011 (cultivated *A. venetum* established in 2011), Alakak, and Hongou (two wild population of *A. venetum*), from 2011 to 2015. DI was calculated by the formula $DI = [100 \times \sum_{i=1}^n (i \times L_n)] / (5 \times LN)$, where i is the disease severity scale ($i = 0, 1, 2, 3, 4$, and 5) and LN and L_n are the total number of leaves and the number of leaves of each disease severity, respectively.

Fig. 5. Disease index (DI) of rust (*Melampsora apocyni*) on three parts of *Apocynum venetum* plants over time in CV2010 (cultivated *Apocynum venetum* established in 2010), CV2011 (cultivated *A. venetum* established in 2011), Alakak, and Honggou (two wild population of *A. venetum*) from 2011 to 2015. DI was calculated by the formula $DI = [100 \times \sum_{i=0}^4 (i \times Ln)] / (5 \times LN)$, where i is the disease severity scale ($i = 0, 1, 2, 3, 4$, and 5) and LN and Ln are the total number of leaves and the number of leaves of each disease severity, respectively. Bars represent standard error of each mean. DI of lower leaves had a significant difference with middle or upper leaves of plant at $P = 0.05$ using the least significant difference test.

total precipitation of 63.4 mm. The mean daily temperature from mid-May to mid-September during these 5 years ranged between 9.8 and 29.8°C (Fig. 1).

Characteristics of topsoil water, vegetation, and *A. venetum* plant. In cultivated plants, there was no significant ($P > 0.05$) change for the relative soil water content, total vegetation coverage, and diversity index across months (Table 1). However, the relative soil water content in wild plants decreased gradually across months (Fig. 2) and showed a significant difference ($P < 0.05$) (Table 1). In contrast, the total vegetation coverage in wild plants increased significantly across months ($P < 0.05$) (Fig. 2; Table 1). No significant ($P > 0.05$) change was observed for the density of cultivated *A. venetum* across months, whereas the density of wild *A. venetum* increased significantly ($P < 0.05$) across months (Fig. 2; Table 1). With the exception of the relative soil water content in wild plants, these variables related to topsoil water, vegetation, and *A. venetum* in cultivated and wild plants showed significant ($P < 0.05$ or 0.01) differences across years (Table 1). For cultivated plants, the relative soil water content did not show a significant ($P > 0.05$) difference between CV2010 and CV2011 in each month, while the total vegetation coverage and the density of *A. venetum* of CV2010 in each month was greater than that of CV2011 ($P < 0.05$) (Fig. 2). The relative soil water content and the density of *A. venetum* of wild plants in Honggou was greater than that of Alakak in each month ($P < 0.05$) (Fig. 2).

The mean relative soil water content in the periods of rust development of cultivated plants in 2011 and 2013 were 69.2 to 81.1% whereas, in 2012, the values were only 57.2 to 65.1%. Except for 2013, the mean relative soil water content of CV2011 was higher than that of CV2010 ($P = 0.034$ and 0.031) (Table 2). In wild plants, the mean relative soil water content during the period of rust development across years showed no significant differences ($P > 0.05$) (Table 2), and the mean relative soil water content was lower than that of cultivated plants in 2011 and 2013. The mean values of total vegetation coverage in CV2010 were higher than that of CV2011 from 2011 to 2013 ($P = 0.013$, 0.011 , and 0.004 , respectively). The mean values of the total vegetation coverage in Honggou were greater than that of Alakak, and the difference was significant ($P < 0.05$) in each year (Table 2). Except for 2012, the mean diversity index of CV2010 was greater than that of CV2011 ($P < 0.001$ and $P = 0.028$ in 2011 and 2013, respectively). In 2011 and 2012, the mean diversity index of Alakak was greater than that of Honggou ($P = 0.013$ and 0.046 , respectively). In the remaining years, the mean diversity index between both fields had no significant difference ($P > 0.05$) (Table 2). The mean density of *A. venetum* in CV2010 and Honggou were higher than that of CV2011 and Alakak in most years ($P < 0.05$) (Table 2).

Urediniospore concentration in air. Urediniospores were not captured by samplers prior to disease onset in the plots of cultivated and wild plants. The urediniospore concentration increased gradually along with DI (Fig. 3). The maximum and accumulated urediniospore concentrations in cultivated plants in 2012 were less than those of 2013 (Fig. 2; Table 2). The maximum and accumulated values in wild plants in 2012 and 2013 were higher than those of 2014 and 2015 (Fig. 3; Table 2).

Assessment of rust development. Rust in cultivated plants usually first occurred from late May to early June. In contrast, wild plants did not exhibit rust symptoms until early June or July (Fig. 4). The DI of cultivated and wild plants gradually increased with time until it reached maximum values (Fig. 4). In cultivated plants, DI_{max} in 2012 remained stable until the plants died naturally (early September), whereas DI_{max} in 2011 and 2013 appeared earlier (early July) than in 2012. In wild plants, DI_{max} from 2012 to 2015 remained until the plants died naturally (from the end of August to early September), whereas DI_{max} in 2011 appeared earlier than in the other years (Fig. 4).

In cultivated plants, DI_{max} ranged from 49.4 to 57.1% from 2011 to 2013, and DI_{max} in wild plants ranged from 6.4 to 75.6% across

2011 to 2015, respectively (Fig. 4). DI_{max} in cultivated plants in 2011 and 2013 was greater than in 2012 ($P < 0.05$), and DI_{max} values in wild plants in 2011, 2012, and 2013 were greater than the values for other 2 years ($P < 0.05$) (Fig. 4). In cultivated plants, in 2011, DI_{max} of CV2010 was lower than that of CV2011 ($P = 0.039$) whereas, in 2012 and 2013, DI_{max} of CV2010 was greater than that of CV2011 ($P = 0.011$ and 0.031). In wild plants, in 2011, DI_{max} of Honggou was greater than that of the Alakak ($P = 0.014$), whereas the values of Honggou were lower than those of Alakak in 2012, 2014, and 2015 ($P = 0.012$, $P = 0.047$, and $P < 0.001$, respectively) (Fig. 4).

DI at different parts of plant. In cultivated plants, the observed dates of rust on the middle leaves were 6 to 7 days and 13 to 29 days later than for the lowest leaves for CV2010 and CV2011, respectively, while rust occurred on the uppermost leaves 7 to 39 days and 7 to 46 days later, respectively, compared with the lowest leaves (Fig. 5). In wild plants, the delayed onset of infection was 5 to 9 days and 7 to 17 days for Honggou and Alakak, respectively, on the middle leaves, while the observed delay was 1 to 14 days and 5 to 28 days, respectively, on the upper leaves (Fig. 5).

In cultivated plants, DI of the middle or upper leaves was less than that of the lowest leaves in the entire disease assessment process. DI of the middle and upper leaves were 39.4 to 53.4% and 52.3 to 72.9% lower, respectively, than those of the lowest leaves ($P < 0.05$) (Fig. 5). However, for wild plants, despite DI of the upper leaves being 31.2 to 94.7% less than those of the lowest leaves, the difference had statistical significance only in the later stage of this disease ($P < 0.05$) (Fig. 5).

Relationships between DI_{max} and its influencing factors.

DI_{max} of cultivated plants was significantly positively correlated with the accumulated precipitation ($r^2 = 0.461$, $P = 0.024$), topsoil water content ($r^2 = 0.891$, $P = 0.003$), total vegetation coverage ($r^2 = 0.558$, $P = 0.008$), *A. venetum* density ($r^2 = 0.549$, $P = 0.009$), and accumulated urediniospore concentration ($r^2 = 0.716$, $P = 0.004$) (Table 3). DI_{max} of wild plants was also significantly positively correlated with the accumulated precipitation ($r^2 = 0.805$, $P = 0.003$), total vegetation coverage ($r^2 = 0.445$, $P = 0.004$), *A. venetum* density ($r^2 = 0.353$, $P = 0.026$), and accumulated urediniospore concentration ($r^2 = 0.749$, $P = 0.004$) but not for the topsoil water content. In addition, the latter showed a significant negative correlation with the species diversity index ($r^2 = -0.367$, $P = 0.020$) (Table 3).

In wild plants, the accumulated precipitation showed a significant positive correlation with the total vegetation coverage ($r^2 = 0.401$, $P = 0.010$) and *A. venetum* density ($r^2 = 0.457$, $P = 0.009$) (Table 3); however, this was not the case in cultivated plants (Table 3). Whether in cultivated or wild plants, the accumulated precipitation did not show a significant ($P > 0.05$) positive correlation with the topsoil water content (Table 3). However, in both cultivated and wild plants, *A. venetum* density showed a significant positive correlation with the total vegetation coverage ($r^2 = 0.829$, $P = 0.002$ and $r^2 = 0.878$, $P = 0.001$, respectively). The accumulated urediniospore concentration showed a significant negative correlation with the daily average temperature ($r^2 = -0.632$, $P = 0.007$ and $r^2 = -0.308$, $P = 0.027$) (Table 3) in both cultivated and wild plants, respectively.

Analysis of the variations in DI_{max} . In cultivated and wild plants, the GOF of PLS-PM were 0.72 and 0.71, respectively, and the models explained 80.0 and 70.1% of the variations in DI_{max} , respectively (Fig. 6A and B).

The CC of cumulative precipitation (CC = 0.990 and 0.969) for the weather factor in both cultivated and wild plants, respectively, were greater than that of daily average temperatures (CC = 0.038 and -0.085, respectively) (Fig. 6A and B). The topsoil water content in cultivated plants and the total vegetation coverage in wild plants had greater contribution (CC = 0.681 and 0.989, respectively) to the microclimatic factor compared with other dominated manifest variables (Fig. 6A and B). In both cultivated and wild plants, the

contribution of the *A. venetum* density (CC = 0.989 and 0.894, respectively) to the host factor exceeded that of the species diversity (CC = 0.072 and -0.154) (Fig. 6A and B). Microclimatic and weather factors in cultivated and wild plants, respectively, had a greater effect (PC = 0.831 and PC = 0.544, respectively, both $P < 0.001$) on $DI_{\max}$ than the other three factors (Fig. 6A and B). In cultivated plants, the weather factor had a significant (PC = 0.313, $P < 0.05$) direct effect on $DI_{\max}$ (Fig. 6A). However, in wild plants, the weather significantly (PC = 0.862, $P < 0.001$) affected the microclimate via the host factor (PC = 0.579, $P < 0.001$) (Fig. 6B), and this indirect process ultimately resulted in a variation of $DI_{\max}$ (mean effect size = 0.119, 95%CI > 0) (Table 4). Despite the host factor remaining unaffected by the weather in cultivated plants (Fig. 6A), it had a significant (mean effect size = 0.699, 95%CI > 0) influence on $DI_{\max}$ via microclimatic factor (Table 4). Furthermore, the host factor also significantly and positively (PC = 0.705, $P < 0.001$) affected inoculum, and the effect was greater than that of wild plants (PC = 0.130) (Fig. 6A and B). However, the direct effect of inoculum on $DI_{\max}$ had no statistical significance ($P > 0.05$) whether in cultivated or wild plants (Fig. 6A and B).

DISCUSSION

In both cultivated and wild *A. venetum* plants, there was considerable year-to-year heterogeneity in rust intensity ($DI_{\max}$) but there was no evidence of a significant correlation between rust intensity and daily average temperature. Moreover, temperature had a very small contribution to the overall weather conditions fostering rust intensity. This may initially be considered surprising given that others (Chandrashekar and Heather 1981; Widin and Schipper 1980) have reported that various components of the *Melampsora* pathogen life cycle (e.g., uredinia density and spore production on leaves) are dependent on temperature. However, during the period of rust development, the temperature in Altay Prefecture was 10.6 to 28.8°C over the 5-year period, which was favorable for the germination of *Melampsora* urediniospores and the resulting infection of leaves (Guo et al. 2010; Li et al. 2013). Thus, temperature may not vary sufficiently from year to year within this location to achieve a detectable level of association with rust intensity. In fact, in the absence of wetness (e.g., rain) or high atmospheric moisture levels (e.g., high relative humidity), temperature alone may be relatively unimportant for plant disease (Kriss et al. 2010). In the current study, the temperature in the rainy weather was 10.6 to 20.3°C, which was still within the optimum range for rust development. Overall, temperature was not a limiting

factor for rust development in both cultivated and wild *A. venetum* in Altay Prefecture, China.

In contrast to temperature, the current study highlighted a strong influence of precipitation on rust development, with differences in precipitation frequency and cumulative amounts responsible for the year-to-year variations in rust intensity. For example, in 2011, 2012, and 2013, DI began to increase continuously after heavy rain or frequent rain events, and the $DI_{\max}$ in these years greatly exceeded that in the poor rainfall years of 2014 and 2015. This was not surprising, because precipitation is a known driver of disease occurrence and development not only with regard to rust caused by the genus *Melampsora* (Covarelli et al. 2013; Lawrence et al. 2007; Widin and Schipper 1980) but also for rust on other crops such as soybean (*Glycine max*) rust (*Phakopsora pachyrhizi*) (Schmitz and Grant 2009) and wheat stripe rust (*Puccinia glumarum*) (Eversmeyer and Kramer 2000; Zeng and Luo 2006).

In cultivated plants, the topsoil was always kept wet due to the application of drip irrigation, thus concealing any significant relationship between the soil water content and precipitation. Importantly, this continuously wet topsoil led to an increased rust intensity as a consequence of its effect on the microclimatic factor, and the extent of this influence exceeded the influence of weather. In addition, variations in timing and intensity of rust occurrence among three parts of cultivated plants were also found in the entire rust development progress. In particular, rust on the lowest leaves was also most influenced by the topsoil water content (e.g., rust first developed on the lowest leaves, and DI of the lowest leaves exceeded that of middle and upper leaves). These findings that highlight the importance of the topsoil water content in determining the rust intensity are supported by other rusts in some cultivated crops that use irrigation such as soybean, coffee (*Coffea arabica*), sugar cane cultivars (*Saccharum* spp.), and blackberry (*Rubus laciniatus*). These studies showed that irrigation is known to increase the topsoil water content and changes the microclimate within the plant canopy, including by increasing the duration of leaf wetness and by reducing canopy temperatures, thus increasing rust intensity (e.g., severity). Such effects were most pronounced for the lowest leaves, and resulted in higher rust intensity than in the upper leaves (de Paiva Custódio et al. 2014; Johnson and Mahaffee, 2010; Sikora et al. 2014; Taylor 1992). Another study found that, even though drip irrigation was applied to plants, the coffee rust (*Hemileia vastatrix*) intensity was not affected by the amount of irrigation, because the topsoil remained dry due to the burying of drip emitters at a depth of 0.25 m in the soil, within the root zone (Paiva et al. 2011). This suggests a potential management

TABLE 3. Spearman's rank correlation matrix between the maximum disease index of rust (*Melampsora apocyni*) and the factors influencing rust (*Melampsora apocyni*) intensity in cultivated and wild *Apocynum venetum* plants^a

Plants, parameters	DI	Temp	Precip	Soil water	Coverage	Density	Ured
Cultivated							
Temp	0.128	...	...	...	...	...	...
Precip	0.461*	0.059	...	...	...	...	...
Soil water	0.891**	-0.051	0.267	...	...	...	...
Coverage	0.558**	0.106	-0.048	0.691**	...	...	...
Density	0.549**	0.041	-0.106	0.744**	0.829**	...	...
Ured	0.716**	-0.632**	0.632**	0.807**	0.761**	0.772**	...
Diversity	0.273	0.456*	0.198	0.195	0.427**	0.083	-0.097
Wild							
Temp	-0.193	...	...	...	...	...	...
Precip	0.805**	-0.102	...	...	...	...	...
Soil water	0.152	-0.208	0.163	...	...	...	...
Coverage	0.445**	-0.083	0.401*	0.551**	...	...	...
Density	0.353*	-0.121	0.457**	0.426**	0.878**	...	...
Ured	0.749**	-0.208	0.850**	-0.100	0.020	0.013	...
Diversity	-0.367*	0.047	-0.341*	-0.127	-0.487**	-0.561**	0.298

^a Abbreviations: DI = disease index, Temp = daily average temperature, Precip = accumulated precipitation, Soil water = relative topsoil water content, Coverage = total vegetation coverage, Density = *A. venetum* density, Ured = accumulated urediniospore concentration, and Diversity = species diversity index. Asterisks * and ** = significance level at $P < 0.05$ and 0.01, respectively.

strategy for improving rust management on cultivated *A. venetum* plants by reduction the topsoil water content through optimizing irrigation and possibly burying drip emitters underground. In wild plants, the topsoil water content did not significantly correlate with the rust intensity as a consequence of increased dry topsoil compared with cultivated plants during the period of rust development. As such, if considering reduction of rust in cultivated plants by optimizing irrigation, the relative water content of topsoil (0- to 20-cm depth) should decrease to below 65% or lower.

Studies on native grass species such as creeping bentgrass (*Agrostis stolonifera*) and small pasque flower (*Pulsatilla pratensis*) and on trees such as the willow (*Salix* spp.) have confirmed the role of increased total vegetation coverage (or canopy density) for reducing air movement and increasing leaf shading. These changes,

in turn, lead to changes of the microclimate within the plant canopy. Such changes include an increase of leaf wetness duration and reduce the temperature and light intensity, conditions that are favorable for rust development (Liu et al. 2016; Toome et al. 2010; Wennström and Ericson 1991). This agrees with the findings of the current study, where total vegetation coverage in either wild or cultivated plants correlated positively with DI_{max} . However, the effect of total vegetation coverage on the microclimate was greater in wild plants than in cultivated plants. This effect seems to be caused by tall weeds in wild plants, including *Phragmites communis*, *Achnatherum splendens*, and *Euphorbia* spp. that always grow alongside *Apocynum venetum* plants. These plants form a tall mixed-plant community, unlike the mostly small-plant community in cultivated plants fields where species of the families

Fig. 6. Partial least squares path modeling (PLS-PM) to evaluate the effects of various factors on the disease intensity for **A**, cultivated and **B**, wild *Apocynum venetum*. Rectangles and ovals represent measured variables and latent variables, respectively. These are connected by arrows showing directed cause-and-effect relationships. Numbers above arrows are contribution coefficients of measurement variables to the latent variables. Numbers within arrows are standardized path coefficients. Pathways without significant effects ($P > 0.05$) are indicated by dashed arrows. R^2 values associated with response variables indicate the proportion of variation explainable by relationships with other variables. Asterisks *, **, and *** indicate significance levels at $P < 0.05$, 0.01, and 0.001, respectively.

Chenopodiaceae and Poaceae grew between rows and formed an independent plant community between the relatively large *A. venetum* plants. This lowers the density of the canopy of cultivated plants relative to that of the wild plants even under the same total vegetation coverage.

Increasing the host density in wheat (*Triticum aestivum*) cultivar mixtures (Chester 1946) and in a natural population of jewelweed (*Impatiens capensis*) (Lively et al. 1995) did not affect the rust intensity by changing the host–pathogen encounter rates when the total vegetation coverage of plant communities remained constant. In contrast, controlled-density studies in monocropping systems such as wheat indicated that rust intensity increased with density, and further indicated that the microclimatic conditions within the plant canopy were more conducive to rust infection because the total vegetation coverage of a single-plant system became higher with increasing plant density (Pfleeger and Mundt 1998). In the current study, the changes of *A. venetum* density in both cultivated and wild plants not only affected the microclimate via the total vegetation coverage but also affected the amount of captured *M. apocyni* urediniospores (inoculum), resulting in a corresponding variation of rust intensity. However, an abundance of taller weeds adjacent to *A. venetum* in wild plants reduced the host–pathogen encounter rates, leading to decreased inoculum with consequently reduced rust. Mitchell et al. (2002) also reported a negative correlation between rust severity (defined as percentage of leaf area infected by a single disease) and plant species diversity in grasslands for *Puccinia* spp. on *Euphorbia corollata*. In contrast, other studies have shown that species diversity has little influence on rust epidemics in situations where weather and other environmental conditions are conducive to the development of rust (Burdon et al. 2006). In the current study, the species diversity of Honggou was higher in 2013 than that of other years, yet DI_{max} was also greater due to abundant rainfall. In addition, the low-height weeds in cultivated plants had no significant effects on the amount of inoculum because they were a virtually independent plant community that provided little obstruction for the movement of *M. apocyni* urediniospores between the larger *A. venetum* plants. Accordingly, this explains why the extensive presence of diverse plants other than *A. venetum*, whether in cultivated or wild plants, did not always result in reduced rust intensity in the current study.

The concentration of *M. apocyni* urediniospores is in line with rust development in cultivated and wild plants. This indicates that the production of reinfecting inoculum mainly originated from local plants and had been produced continually on infected leaves. This ensures the potential for rust development in local plants as soon as the environmental conditions were conducive. In fact, with regard to rust, except for alloinfection (defined as infection in which the infector host is a different individual from the infected host

individual), disease development is mainly caused by urediniospores spreading in air (Mundt 2009). High rates of autoinfection (defined as infection resulting from inoculum produced on the same host unit) contribute in a major way to rust development at small spatial scales (Mundt 2009). For example, autoinfection can cause more than 50% of the total stem rust infection in a perennial ryegrass (*Lolium perenne*) (Pfender 2006). However, autoinfection is often ignored in disease development because it is challenging to measure directly. In the current study, the effect of inoculum on DI_{max} was insignificant in both cultivated and wild plants. This implies that the viewpoint that inoculum factor is only dominated by the concentration of *M. apocyni* urediniospores in the air may be incorrect. Still, the presence of sufficient quantities of urediniospores spreading through wind is an important factor for rust development. Although there was no direct evidence from historical studies that would indicate where the initial inoculum of rust had come from, the current study indicates that the production of the inoculum to initiate rust in either cultivated or wild plants most likely originated from local plants because no urediniospores were captured by air sampling until rust was observed. An understanding of the life cycle of *M. apocyni*, including how this pathogen overwinters when the stems die at the start of winter, would further contribute to a more comprehensive understanding of the rust development of *A. venetum*. Such knowledge may also indicate additional ways to reduce its damaging effects. Recently, Barua et al. (2018) investigated how some epidemics commence without the obvious presence of “alternate” or “green bridge” hosts for the stem rust pathogen *Puccinia graminis* f. sp. *tritici*, and found that urediniospores can survive in the absence of suitable host plants for up to a year and still germinate and successfully initiate infections of wheat seedlings. Hence, it is possible that similar survival capabilities apply for the rust cycle of *A. venetum* in China, and this would be worthwhile to investigate further.

In conclusion, we found considerable variations in rust intensity in both cultivated and wild plants across years, plant vertical direction, and sample sites, and clarified that precipitation plays a key role in the variation of year-to-year rust intensity. The topsoil water content and total vegetation coverage in cultivated and wild plants, respectively, were the critical determinants of rust intensity due to their effects on microclimate. For cultivated and wild plants, increasing *A. venetum* density not only increased the total vegetation coverage but also could increase the host–pathogen encounter rates, thus resulting in the rust becoming more severe. However, weeds in wild plants could alleviate *A. venetum* rust by reducing the host–pathogen encounter rates in years with less rain. We believe that this study provides novel information for *A. venetum* rust research. The presented results can be used to forecast and to control rust development in future.

TABLE 4. Indirect effects of various factors on the maximum disease index of rust (*Melampsora apocyni*) in the cultivated and wild *Apocynum venetum* plants^a

Pathway to DI ^b	Effect	SE	95%CI	
			Lower	Upper
Cultivated plants				
Weather factor → Microclimatic factor → DI	0.186	0.183	−0.324	0.480
Weather factor → Host factor → Microclimatic factor → DI	0.052	0.194	−0.354	0.401
Weather factor → Host factor → Inoculum factor → DI	−0.010	0.044	−0.114	0.070
Host factor → Microclimatic factor → DI	0.699	0.140	0.381	0.963
Host factor → Inoculum factor → DI	0.115	0.123	−0.122	0.366
Wild plants				
Weather factor → Microclimatic factor → DI	−0.001	0.028	−0.057	0.060
Weather factor → Host factor → Microclimatic factor → DI	0.119	0.069	0.016	0.268
Weather factor → Host factor → Inoculum factor → DI	0.017	0.019	−0.014	0.063
Host factor → Microclimatic factor → DI	0.200	0.107	0.018	0.410
Host factor → Inoculum factor → DI	0.027	0.029	−0.024	0.091

^a CI = confidence interval, Effect = mean effect size, and SE = standard error.

^b DI = disease intensity.

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