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Title: Temporal sporulation of *Thekopsora areolata* and *Chrysomyxa* spp. in Finnish Norway spruce seed orchards

Year: 2021

Version: Published version

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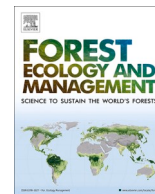
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Please cite the original version:

Kaitera J., Aarnio L., Karhu J., Ylioja T. (2021). Temporal sporulation of *Thekopsora areolata* and *Chrysomyxa* spp. in Finnish Norway spruce seed orchards. *Forest Ecology and Management* 499, 119557. <https://doi.org/10.1016/j.foreco.2021.119557>.

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Temporal sporulation of *Thekopsora areolata* and *Chrysomyxa* spp. in Finnish Norway spruce seed orchards

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ARTICLE INFO

Keywords:

Aecia
Cherry-spruce rust
Chrysomyxa needle rust
Disease control
Inland spruce cone rust
Rust epidemics

ABSTRACT

Cone rusts *Thekopsora areolata* and *Chrysomyxa pirolata* are widely distributed in northern hemisphere. In Fennoscandia, these rusts cause severe damage and great economical losses especially in seed orchards specialized to produce high quality seeds. The aim of this study was to investigate sporulation of cone rusts and factors affecting epidemics to develop new control practices against these rusts. Natural sporulation of *T. areolata* was investigated in Finnish Norway spruce seed orchards on *Prunus padus* and the effect of environmental variables on rust sporulation in 2018–19. A sample of *Prunus* leaves was collected from which the coverage and number of *T. areolata* pustules were estimated. The frequency of cones with cone rusts, *T. areolata*, *Chrysomyxa pirolata* and *C. ledi*, was also estimated in *Picea abies* cones and the sporulation of *C. pirolata* on *Pyrola* sp. leaves in the seed orchards. The *T. areolata* incidence on *Prunus* was modelled with temperature sum, rainfall, seed orchard and time of estimation.

The *T. areolata* disease incidence was low (<30%) in seed orchards in 2018, but it exceeded 100% in 2019. *T. areolata* pustules covered less than 10% of the leaves in 2018 and 10–40% in 2019. An ascomycete, *Phloeosporella padi*, was common in all *Prunus* leaves in 2018–19. Low rainfall in May–June explained the low disease rate on *Prunus* in 2018, while high rainfall explained the high disease rate in May–June in 2019. Cumulative rainfall, temperature sum, time and their interactions were all significant variables in a disease model explaining the disease incidence, while seed orchard was a non-significant variable. Cone rust frequency was low in *P. abies* cones in the seed orchards in 2019. Telia with basidia of *C. pirolata* were rare in May–June on *Pyrola* due to low rainfall in 2018, but frequent due to high rainfall in 2019. Rust control of *T. areolata* is recommended to concentrate on *Prunus* to reduce the number of overwintering diseased leaves in the seed orchards. Old infected cones should be removed from seed orchards to reduce aeciospore dissemination to *Prunus* in May–June. Control of *C. pirolata* is recommended to concentrate on *Pyrola* in early May.

1. Introduction

The cone production in Norway spruce [*Picea abies* (L.) Karst.] seed orchards is infrequent and irregular flowering of spruce combined with problems caused by fungi and insects have caused shortage of high quality seeds needed for production of spruce seedlings in forest nurseries. Among fungi, especially cone rusts reduce seed crop, cause premature opening and malformation of cones, and reduce seed germination and number of healthy seeds in infected cones (Singh & Carew, 1990; Kaitera & Tillman-Sutela, 2014). Norway spruce cones are infected by three cone rusts: cherry-spruce rust, *Thekopsora areolata* (Fr.) Magnus, and inland spruce cone rust, *Chrysomyxa pirolata* Wint., cause

significant damage on *Picea* widely over northern hemisphere (Savile, 1950; Ziller, 1974; Gäumann, 1959). The third cone rust, *Chrysomyxa ledi* (Alb. & Schw.) de Bary, sporulates on *Picea* cone scales (Kaitera et al., 2010, 2014, 2017), but it does not affect seed development (Kaitera & Tillman-Sutela, 2014).

The alternate hosts of *T. areolata* belong to the genus *Prunus* (Gäumann, 1959). When *Prunus* leaves are infected, disease symptoms of *T. areolata* appear as rust pustules with red to violet colour (Kaitera et al., 2019). *Thekopsora areolata* has five spore stages: first the monokaryotic basidiospores develop on overwintered *Prunus* leaves from germinating teliospores and infect pistillate cones of *Picea abies* in the spring and early summer. In infected cones, spermatogonia develop first

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and after spermatization the dikaryotic mycelium forms dikaryotic aecia. Aeciospores spread from aecia to infect *Prunus* leaves, where dikaryotic uredinia and telia develop (Gäumann, 1959). The rust spreads among *Prunus* by urediniospores. *Chrysomyxa pirolata* Wint. has similarly five spore stages, but the rust spreads via *Moneses*, *Pyrola* and *Orthilia* (Ziller, 1974). On overwintered *Pyrola* leaves, the rust forms first in the spring undifferentiated fruiting stages that develop either to uredinia or telia depending on the available precipitation (Crane & Hiratsuka, 2000). Basidiospores develop on telia and spread to cones of *P. abies*, where spermogonia and aecia develop. Aeciospores from aecia then infect leaves of *Pyrola* (Gäumann, 1959). *Chrysomyxa ledi* overwinters as telia on *Ledum* spp., from which basidiospores spread to needles and cones of *Picea* spp., where spermogonia and aecia develop. Aeciospores then infect leaves of *Ledum* spp., where uredinia and telia develop (Gäumann, 1959; Kaitera et al., 2010, 2017).

The intensity of *T. areolata* has not been investigated on *Prunus* before. Besides *T. areolata*, other fungi can also sporulate on *Prunus* leaves in northern Europe. Such species are an ascomycete *Blumeriella jaapii* Rehm Arx [anamorph *Phloeosporrella padi* (Lib.) v. Arx (syn. *Cylindrosporium padi* (Lib.) P. Karst Ex. Sacc.)] that causes cherry leaf spot (Khan et al., 2016), *Polystigma ochraceum* (Whal.) Sacc., *P. rubrum* (Pers.) D.C. (Ghazanfari and Banihashemi, 1976), *P. fulvum* Pers. ex DC., *Podosphaera tridactylla* (Wallr.) de Bary and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers and Phillips. *Stigmata carpophila* (Lév.) M.B. Ellis causes also holes in *Prunus* leaves, but it does not sporulate on the leaves. *Blumeriella jaapii* overwinters in dead leaves of *Prunus*, where the sexual stage, apothecia, develop and infect young leaves with ascospores. Infected leaves carry purple spots that turn brownish and later blackish. Secondary infection of leaves takes place by asexual conidia from acervuli on the leaves (Khan et al., 2016).

Fruiting and sporulation of rusts are strongly affected by climatic factors like precipitation and temperature. Optimal range for aeciospore germination of *T. areolata* is 15–24 °C, but the rust can germinate already at 6 °C in the spring (Kaitera & Karhu, 2021). No other studies about the effect of temperature and precipitation on *T. areolata* are available, but among *Cronartium* rusts, the range of germination of aeciospores of *Cronartium pini* (Willd.) Jøst is 5–30 °C, of urediniospores 5–25 °C and of basidiospores 10–25 °C on agar (Ragazzi et al., 1986), while on plant leaves the range of germination of both aeciospores and urediniospores is 15–25 °C (Ragazzi et al., 1989). The range of germination of aeciospores of *C. ribicola* J. C. Fisch is 8–24 °C and of urediniospores 16–28 °C (Van Arsdell et al., 1956). The germination range of aeciospores of *C. fusiforme* Hedgcock & Hunt ex Cummins is 11–29 °C and of urediniospores 8–29 °C (Siggers, 1947). On oak leaves, uredinia of *C. quercuum* f. sp. *fusiforme* (Hedgc. & N. Hunt.) Bursdall & G. Snow develop most frequently at 9–18 °C (Kuhlman, 1987). Disease establishment of *C. pini* on *Vincetoxicum hirundinaria* Medicus is affected also by light, precipitation and age of the plant leaves (Ragazzi, 1983, 1992). Under wet conditions young uredinia of *C. pirolata* develop into telia, but telia formation is also affected by temperature (Crane & Hiratsuka, 2000). Rain also affects telia formation and basidiospore release from *Pyrola* to *Picea mariana* (Mill.) Britton, Sterns & Poggenburg (Singh and Carew, 1990). In Finland, uredinia development and sporulation of *C. pirolata* on *Orthilia secunda* (L.) House increased along rising temperature and rainfall. Intense periods with rainfall over 10 mm per day in late May coincided with formation of telia and basidia, when temperature sum exceeded 100 d.d. Telia were formed, when cumulative rainfall exceeded 150 mm (Kaitera & Tillman-Sutela, 2015).

Disease control of cone rusts is inadequately studied. Nowadays prohibited fungicide in EU decreased infection by *C. pirolata* (Summers et al., 1986). Later other fungicides have not been tested and chemical control of *T. areolata* is not practiced at seed orchards. Recently, the heteroecious nature of the rust was shown that excluded possibility of the rust to spread from spruce to spruce (i.e. from cone to cone) via aeciospores (Kaitera et al., 2019). Therefore, control of *T. areolata* at seed orchards was recommended to be targeted to *Prunus*.



Fig. 1. Locations of investigated Norway spruce seed orchards in southern Finland. 1 = Heinämäki 170, 2 = Paronen 365, 3 = Sillanpää 235, 4 = Imatra 374, 5 = Suhola 403, 6 = Riihimäki 169, 7 = Palvaanjärvi 172, 8 = Taavetti 428, 9 = Metsä-Ihala 176.

The aim of this study was to 1) investigate the seasonal sporulation of *T. areolata* on wild *Prunus* and Norway spruce to describe the mode of spreading of the rust in Finnish Norway spruce seed orchards. The purpose of the study was also to 2) improve rust control in seed orchards based on epidemical and weather information. In addition, it was also aimed to 3) improve control of *C. pirolata* based on rust sporulation on *Pyrola* and *Picea abies*. Additionally, it was aimed to 4) describe rust symptoms of *T. areolata* and another ascomycete on *Prunus* leaves to improve rust diagnostics of these pathogens on the leaves. Factors affecting rust epidemical patterns were also discussed.

2. Materials and methods

2.1. Selection of sample trees

Sixteen *Prunus padus* trees were selected within five Norway spruce seed orchards (nos. 1–5; Nikkanen et al., 1999) that had suffered from continuous *T. areolata* epidemics in southern Finland during the 2000s (Fig. 1). In addition, three sample trees were selected ca. 1–3 km away from three seed orchards (nos. 6–8, Fig. 1) that lacked *Prunus* trees inside the seed orchards. The sample trees were selected at the time of *Prunus* flowering in May from each seed orchard. In general, the seed orchards were surrounded by stands of Scots pine (*Pinus sylvestris* L.), Norway spruce or silver birch (*Betula pendula* Roth) and some had agricultural field neighboring one of the edges.

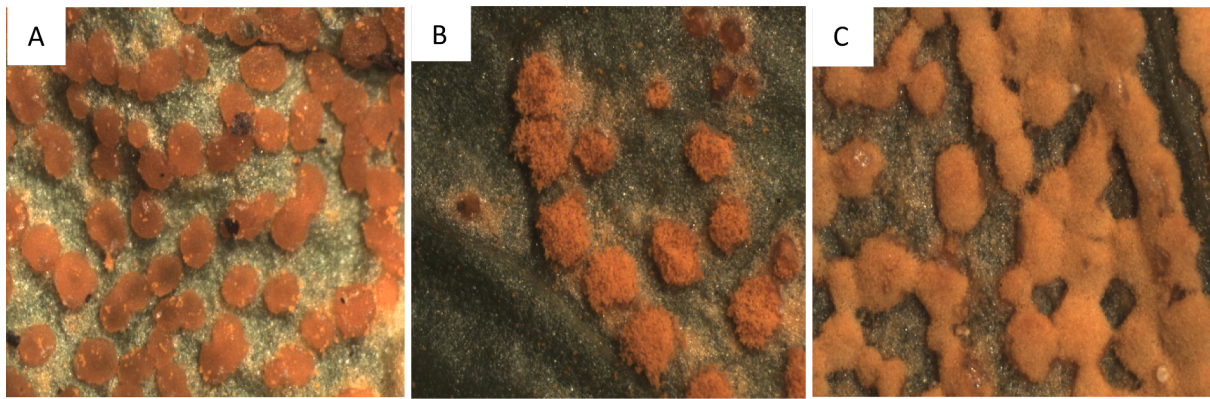


Fig. 2. Estimated fruiting stages of *Chrysomyxa pirolata*: A. undifferentiated fruiting stages, B. uredinia and C. telia with basidia on *Pyrola* sp.

2.2. Estimation of disease incidence of *T. areolata* on *Prunus* trees in seed orchards

Disease incidence class of each sample tree was estimated visually in the field as the percentage of infected leaves per tree. The disease rating covered nine classes ‘0%’, ‘1–5%’, ‘5–10%’, ‘10–30%’, ‘30–50%’, ‘50–70%’, ‘70–95%’, ‘95–99%’ and ‘100%’ of infected leaves per tree. In case at least one leaf showed rust symptoms, the disease rating reached the score ‘1–5%’. The *Prunus* trees were rated during growing seasons in ca. two-weeks’ interval. The sampling dates were May 21–24, June 11–13, July 2–5, July 16–18, July 30 - Aug 1, Aug 13–15, Aug 27–29, Sept 10–12 and Sept 24–27 (9 times) in 2018, while they were May 20–22, June 11–13, June 24–27, July 8–10, July 22–25, Aug 5–7, Aug 19–21 and Sept 2–5 (8 times) in 2019.

Infection of *T. areolata* in *Prunus* leaves was determined as violet (early season), red-brownish (mid-season) or blackish (late season) pustules on the leaves, where hyaline to slightly yellowish uredinia occurred on the lower (abaxial) leaf surface (Gäumann, 1959; Kaitera et al., 2014). Symptoms of *T. areolata* on artificially inoculated *Prunus* leaves (Kaitera et al., 2019) were also used as aid in disease estimation. Brownish pustules without uredinia but with whitish masses of conidia were determined as symptoms of an ascomycete.

2.3. Sampling of *Prunus* leaves

A random sample of ca. 10–50 *Prunus* leaves carrying possible rust symptoms were collected from each sample *Prunus* simultaneously during the disease rating. At the beginning of the season the sample size was higher than in the late season due to shedding of leaves at the end of the season in 2018. In 2019, the sample size was 10–30 leaves. The leaves were collected as dry or air-dried immediately after collection to avoid infection by secondary fungi. The leaves were air-dried in paper bags prior to microscopy in the laboratory.

2.4. Disease variables on the *Prunus* sample leaves

In the laboratory, the coverage area of the violet pustules carrying *T. areolata* uredinia on the lower leaf surface as percentage from the total leaf area was estimated from the sample leaves. This was done using a broad percentage grid as aid. Pustules with hyaline to slightly yellowish to whitish uredinia were identified as *T. areolata* pustules by comparing their morphology to those produced in artificial inoculations and identified genetically (Kaitera et al., 2019). The state of uredinial sporulation was determined by classes ‘sporulation occurs’ or ‘no sporulation’ for the pustules based on whitish to hyaline urediniospores protruding from uredinia through a pore at the top (Kaitera et al., 2019). Also the number of *T. areolata* pustules per leaf was counted.

Besides *T. areolata*, the coverage area percentage of an ascomycete

that formed brownish pustules, in which whitish conidia protruded from acervuli of *Phloeosporrella padi* (Lib.) v. Arx (syn. *Cylindrosporium padi* (Lib.) P. Karst Ex. Sacc.), the anamorph of *Blumeriella jaapii* Rehm. Arx (Khan et al., 2016), was estimated similarly on the lower (abaxial) leaf surface. Leaves were checked in the laboratory using a stereo microscope (Wild). Conidia morphology of *C. padi* was studied by measuring the length and width of 100 acervuli, and length and width of 100 conidia from 5 to 6 sample leaves from 3 to 4 seed orchards under stereo and light (Meiji) microscopes and compared to descriptions in the literature (Khan et al., 2016). Before the microscopy of conidia, the conidia were stained with lactophenol cotton blue.

Thekopsora areolata in all the seed orchards had been identified from aeciospores and urediniospores produced artificially in rust pustules on *Prunus* as *T. areolata* (Kaitera et al., 2019).

2.5. Variation in temperature and rainfall during the sampling periods

Temperature sum (with 5 °C threshold) and cumulative rainfall were both calculated daily starting from May 1st until the last sampling date in September for all the seed orchards in 2018 and 2019. The meteorological observations were collected from the closest grid point of meteorological measurement by the Finnish Meteorological Institute (Venäläinen et al., 1995) next to each seed orchard.

2.6. Estimation of annual sporulation of cone rusts in *Picea abies* cones

In 2019 abundant flowering of spruce allowed to estimate rust occurrence in cones unlike in 2018. Among the young cones, a sample of ca. 60–500 cones per the nine seed orchards (Fig. 1; nos. 1–9) were checked at the same sampling dates (8 times) as the *Prunus* were investigated. In the spruce trees, *Thekopsora* and *Chrysomyxa* fruiting stages were checked from cones in the lower canopy (at below 5 m height from the ground) by pulling down branches bearing cones to eye-level. The first time of observation occurred at the pistillate cone stage. In addition, cones were checked additional four times in one seed orchard (Suhola 403) in mid-June, early July, mid-July and early September. The cones were checked visually for spermogonia and aecia of *T. areolata* (for the description of the fruiting stages, see Roll-Hansen, 1947; Gäumann, 1959; Kaitera et al., 2009, 2014, 2017), of *C. pirolata* (Sutherland et al., 1984; Kaitera et al., 2009) and of aecia of *C. ledi* in cone scales (Kaitera et al., 2010, 2014, 2017). Occurrence of spermogonial stage was determined by the spermatial fluid that oozed out from spermogonia on the cone scale forming a whitish (*T. areolata*) or yellowish (*C. pirolata*) sticky layer of spermatia with a strong odour in droplets. As spermogonia of *C. ledi* are very small (Kaitera et al., 2010) and difficult to see without tearing the cone scales, we did not estimate their presence. A cone was estimated as infected one, if at least one scale bore rust fruiting stages.

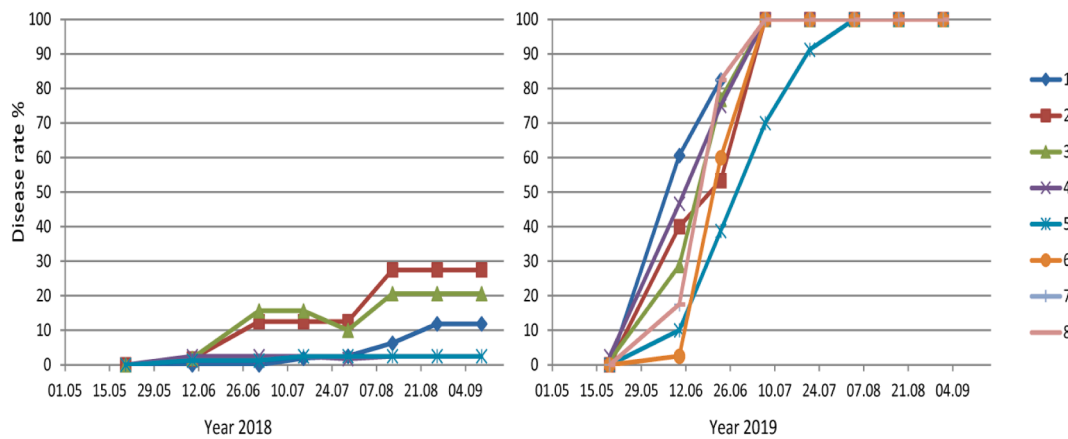


Fig. 3. Temporal variation in average *Thekopsora areolata* disease rate of *Prunus* trees in seed orchards 1–5 in 2018 (left) and 1–8 in 2019 (right). Seed orchards were introduced in Fig. 1.

2.7. Estimation of sporulation of *Chrysomyxa pirolata* on *Pyrola* sp.

Temporal sporulation of *Chrysomyxa pirolata* on alternate hosts was studied in one seed orchard (Paronen 365), where *C. pirolata* has been a serious problem regularly (Savonen, 2001; Kaitera, 2013) due to high frequency of *Pyrola* sp. in the ground vegetation. In the seed orchard, a sample of ca. 30 overwintered *Pyrola* sp. leaves with rust symptoms were collected four times in May–June 2018 and once in June 2019 to determine the quantity and quality of *C. pirolata* fruiting stages over time. The degree of rust sporulation was estimated by the coverages of various spore stages on each infected leaf. The proportion of leaves with different spore stages was also counted. The first estimated spore stages were undifferentiated fruiting stages (Crane & Hiratsuka, 2000; Kaitera & Tillman-Sutela, 2015). Uredinia were determined based on their yellowish colour and typical reticulate urediniospores (Gäumann, 1959; Crane & Hiratsuka, 2000; Kaitera & Tillman-Sutela, 2015). Telia with basidia were determined based on their whitish and fluffy appearance due to sporulation (Sutherland et al., 1984; Crane & Hiratsuka, 2000; Kaitera & Tillman-Sutela, 2015). The spore stages (Fig. 2) were determined on *Pyrola* sp. using a stereo microscope.

2.8. Statistical analysis

Average disease incidence of *T. areolata* was counted between the 16 sample trees in the five and eight seed orchards in 2018 and 2019, respectively. The midpoint of each disease class i.e. average of upper and lower class limits per tree for the five seed orchards were used in statistical analyses. Due to shedding of the leaves in autumn 2018 infection rate data from September 24–27 were not used in the statistical analyses. The average disease incidence of all trees was compared to temperature sum and cumulative rainfall over time visually.

The disease incidence was modelled with seed orchard, cumulative rainfall and temperature sum for both years using the mixed procedure of SAS software (SAS Institute Inc., version 9.4). Dependent variable disease incidence was modelled as continuous proportion from zero to one (or 0% to 100%) with normal distribution after logit transformation. Independent variables tree and year were used as random factors and the independent variables cumulative rainfall and temperature sum as continuous ones. The type III test for fixed effects was used to compare the effect of different variables and their interactions on the disease incidence.

The average coverage of *T. areolata* pustules with uredinia was modelled with seed orchard, time of estimation and interaction between seed orchard and time of estimation, where time of estimation was a continuous variable and seed orchard a factor using the glimmix procedure of SAS software (SAS Institute Inc., version 9.4). Dependent

variable average coverage of *T. areolata* pustules with uredinia was modelled as continuous proportion from zero to one (or 0% to 100%) with beta distribution. In the model, sample tree and year were random variables. The measures within a tree were treated as repeated measures with autoregressive covariance structure so that different correlation estimates were counted separately for different years.

Similarly, the number of uredinial pustules of *T. areolata* was also modelled with seed orchard, time of estimation and interaction between seed orchard and time of estimation. The number of uredinial pustules was modelled using the glimmix procedure of SAS software with normal distribution separately for the two years. In the model, sample tree was a random variable.

Also the average coverage of *P. padi* pustules was modelled with seed orchard, time of estimation and interaction between seed orchard and time of estimation using the glimmix procedure of SAS software (SAS Institute Inc., version 9.4). Dependent variable average coverage of *P. padi* pustules was modelled as continuous proportion from zero to one (or 0% to 100%) with beta distribution. In the model, sample tree and year were random variables.

3. Results

3.1. Temporal variation in *T. areolata* disease incidence on *Prunus* in seed orchards

The disease incidence varied among trees from 0 (class 0) to 50–70% (class 4) between May–September 2018 (not shown). Among the seed orchards, rust incidence was highest in seed orchards 2 and 3 (Paronen 365 and Sillanpää 235), where two trees in both seed orchards had more than 30% disease rate. The average disease incidence was less than 30% in all seed orchards showing that the general disease incidence was low in 2018 (Fig. 3). Among the seed orchards, the disease incidence was less than 5% in two seed orchards, Imatra 374 and Suhola 403. The disease rate was low from May to mid-June, increased slightly until late July and then increased to mid-August, after which it remained constant until the end of September. Rust uredinia appeared first on *Prunus* in early June, and urediniospores were observed on uredinia throughout the growing period.

In 2019, the disease incidence was much higher and the trees were more severely infected than in 2018 (Fig. 3). First uredinia appeared on *Prunus* in late May. The average disease rate was low (<5%) in five trees in late May after which it increased in all trees from mid-June to early July from 30% to 100% in five seed orchards (Fig. 3). In the other three seed orchards, the disease rate reached 100% before late July. As a consequence of the low disease incidence on *Prunus* in 2018, a low number of infected *Prunus* leaves overwintered in 2018. Due to high

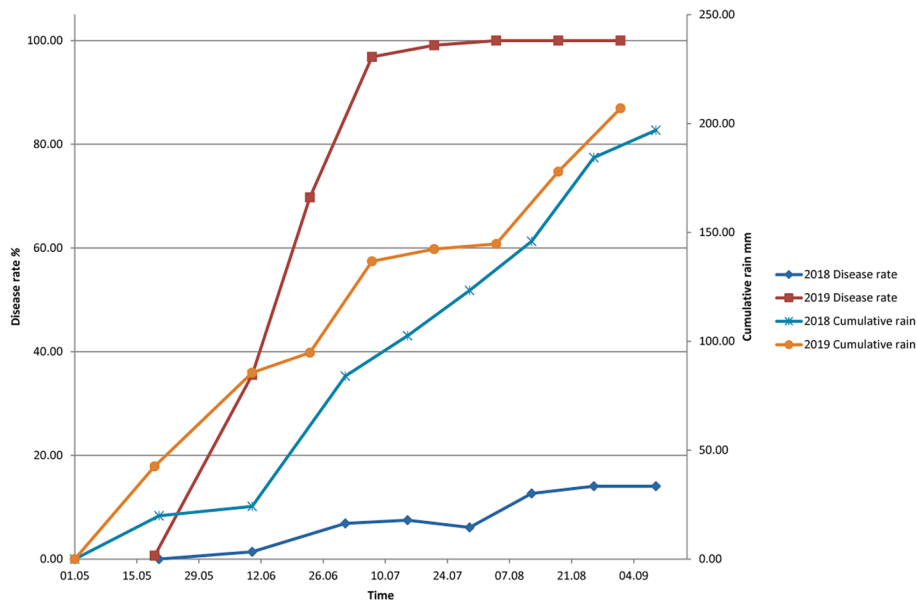


Fig. 4. Relationship between cumulative rainfall and average *T. areolata* disease incidence of *Prunus* trees in seed orchards 1–5 in 2018–19. Seed orchards were introduced in Fig. 1.

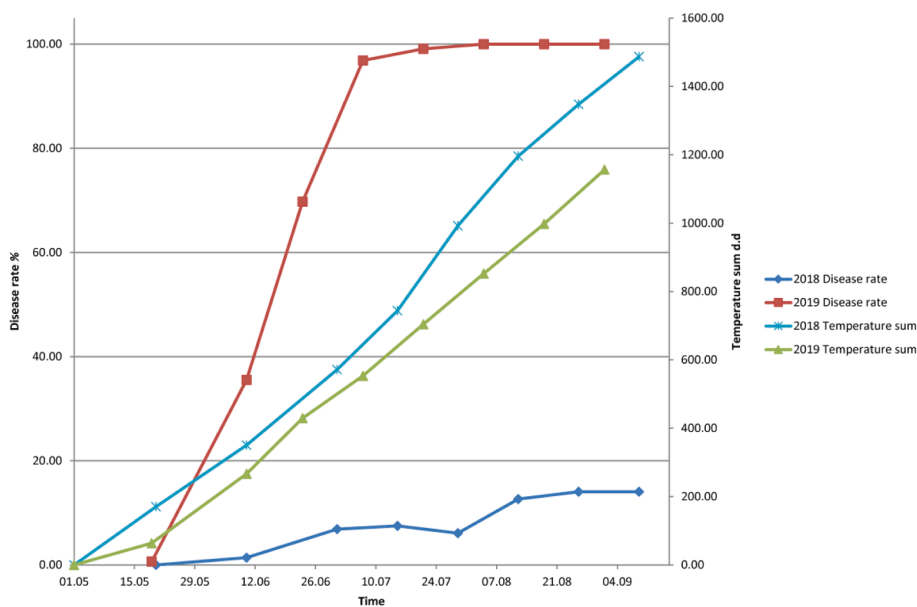


Fig. 5. Relationship between temperature sum and average *T. areolata* disease rate of *Prunus* trees in seed orchards 1–5 in 2018–19. Seed orchards were introduced in Fig. 1.

disease incidence in 2019, abundant number of infected *Prunus* leaves overwintered in 2019. Therefore, the potential basidiospore spreading from overwintered infected leaves was low in the spring 2019 and high in 2020.

When comparing the average disease rate of all trees to cumulative rainfall and temperature sum separately in both years, the dry period from late May to early June did not favour aeciospore infection and further disease progress to uredinia on *Prunus* in 2018. The slight increase in disease rate in July–August coincided with increase in cumulative rainfall, but the effect was low (Fig. 4). In 2019, the rainy period from late May to mid-June favoured aeciospore dissemination and disease progress on *Prunus* leading to high disease incidence in early July. The temperature sum was high during the growing seasons both in 2018 and 2019 already since May (Fig. 5).

When modelling the average disease rate of trees, cumulative rainfall, temperature sum and their interaction were significant variables in a linear mixed model, while seed orchard and interaction between temperature sum and seed orchard were insignificant variables, when year and tree were random variables (Table 1). The model was:

Disease incidence = cumulative rainfall + temperature sum + cumulative rainfall*temperature sum, when excluding the insignificant variables from the model.

In 2019, model for the average disease rate in seed orchards showed that the greatest increase in rainfall from 50 mm to 150 mm (not shown) and in temperature sum from ca. 0 to 200 d.d. (not shown) lead to increase in the disease incidence from 0% to 100% in all five seed orchards. In 2018, increase in cumulative rainfall from 20 mm to 200 mm (not shown) and in temperature sum from 0 to 1500 d.d. (not shown)

Table 1

Test of fixed effects (type III) of the general linear mixed model. Disease incidence = cumulative rainfall + temperature sum + cumulative rainfall*temperature sum on the average *T. areolata* disease incidence of *Prunus* trees in Finnish seed orchards in 2018–19. Year and tree were included as random effects (not shown here).

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Cumulative rainfall	1	150	23.93	<0.0001
Temperature sum	1	124	43.80	<0.0001
Cumulative rainfall * Temperature sum	1	115	53.00	<0.0001
Seed orchard	4	80.4	0.82	0.5162
Cumulative rainfall * Seed orchard	4	165	1.00	0.4098
Temperature sum * Seed orchard	4	143	1.83	0.1263
Cumulative rainfall * Temperature sum * Seed orchard	4	120	1.25	0.2944

lead to increase in disease incidence from 0% to 60%.

3.2. Temporal variation in *T. Areolata* sporulation on *Prunus* sample leaves

First *T. areolata* pustules with uredinia were observed on sample leaves in early June 2018. The average coverage of *T. areolata* pustules per leaf was low (<10%) between early June and late September with slight increase in late August (Fig. 6). Only small variation occurred in the coverage of *T. areolata* pustules between trees within the seed orchards. In 2019, the average coverage of *T. areolata* pustules per leaf was higher than in 2018, varying between 5 and 50%. First uredinia occurred in late May and since that the coverage of such pustules increased to early September. When modelling the average coverage of *T. areolata* uredinial pustules, time of estimation was a statistically significant variable, while seed orchard and interaction between seed orchard and time of estimation were insignificant variables in a model, where year and tree were random variables (Table 2). The used linear

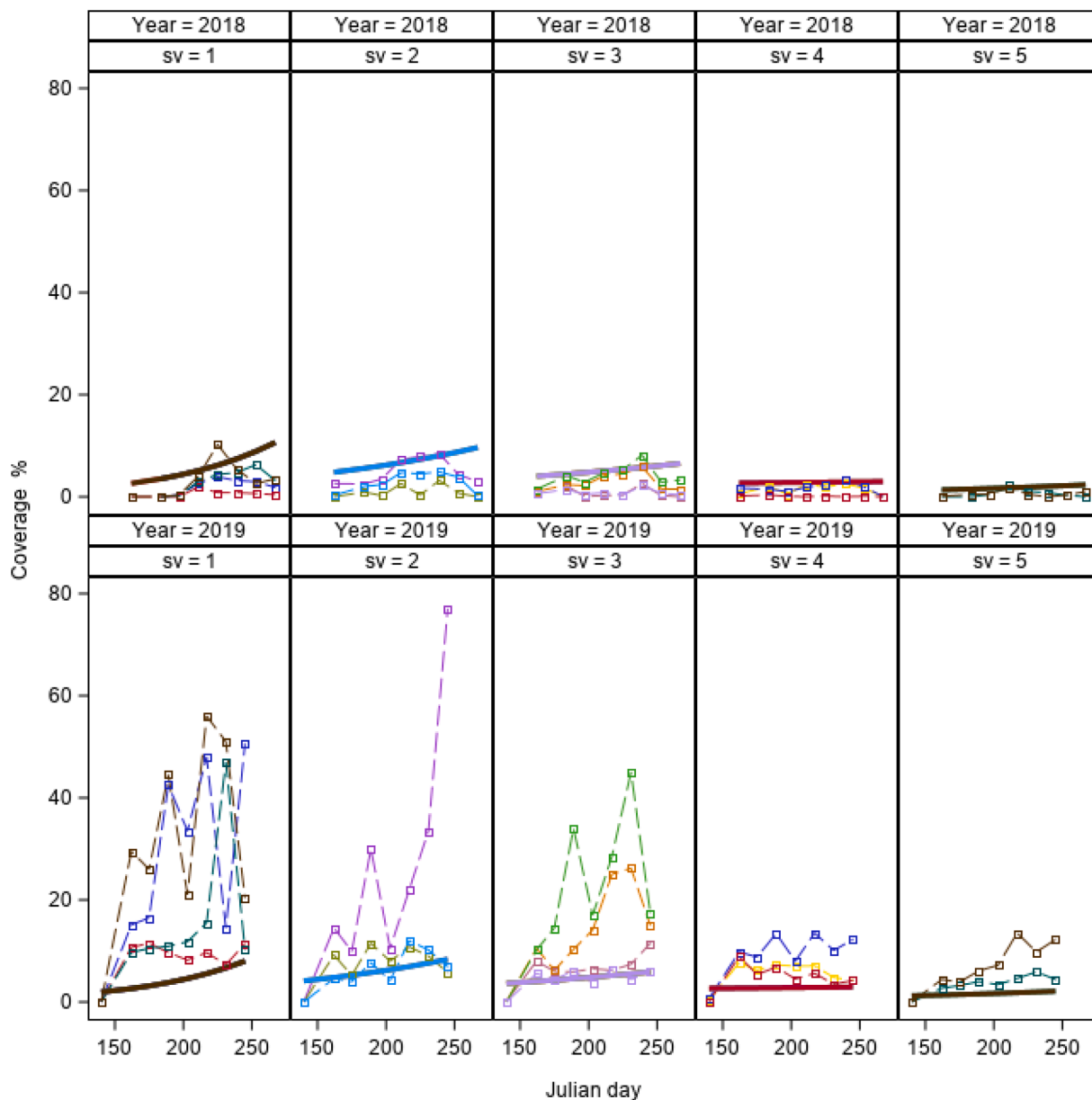


Fig. 6. Model of coverage of *T. areolata* pustules with uredinia per leaf for trees in seed orchards in 2018–19. SV = Seed orchard. Tree observations are marked with dashed lines and the model prediction with a solid line.

Table 2

Test of fixed effects (type III) of the general linear mixed model Coverage of *T. areolata* pustules with uredinia = seed orchard + time of estimation + seed orchard * time of estimation in Finnish seed orchards in 2018–19. Random variables are year and tree.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Seed orchard	4	140.6	0.81	0.5199
Time of estimation	1	125.6	6.48	0.0121
Seed orchard × time of estimation	4	122.6	1.20	0.3134

model was:

Coverage of *T. areolata* pustules with uredinia = seed orchard + time of estimation + seed orchard * time of estimation.

The average number of *T. areolata* pustules with uredinia was low among trees and seed orchards in early June and it continued similarly low until September without any increase in 2018 (Fig. 7). In 2019, the

average number of such pustules per leaf was higher than in 2018. The number of pustules increased from late May to early July and continued to increase until mid-August after which it was reduced until early September. Variation occurred in the number of pustules both between individual trees and seed orchards with a very similar trend (Fig. 7). Uredinia sporulated immediately after uredinia formation in all sample leaves and the sporulation continued similarly throughout the growing period both in 2018 and 2019. When modelling the variation in average number of *T. areolata* pustules separately in 2018 and 2019, time of estimation, seed orchard and their interaction were all insignificant variables in 2018 in a model, where tree was a random variable (not shown). In 2019, time of estimation was, however, a statistically significant variable, while seed orchard and interaction between seed orchard and time of estimation were insignificant variables (not shown). The used linear model was:

Number of *T. areolata* pustules = seed orchard + time of estimation + seed orchard * time of estimation.

Violet colour was detected in the *T. areolata* pustules (Fig. 8a), where

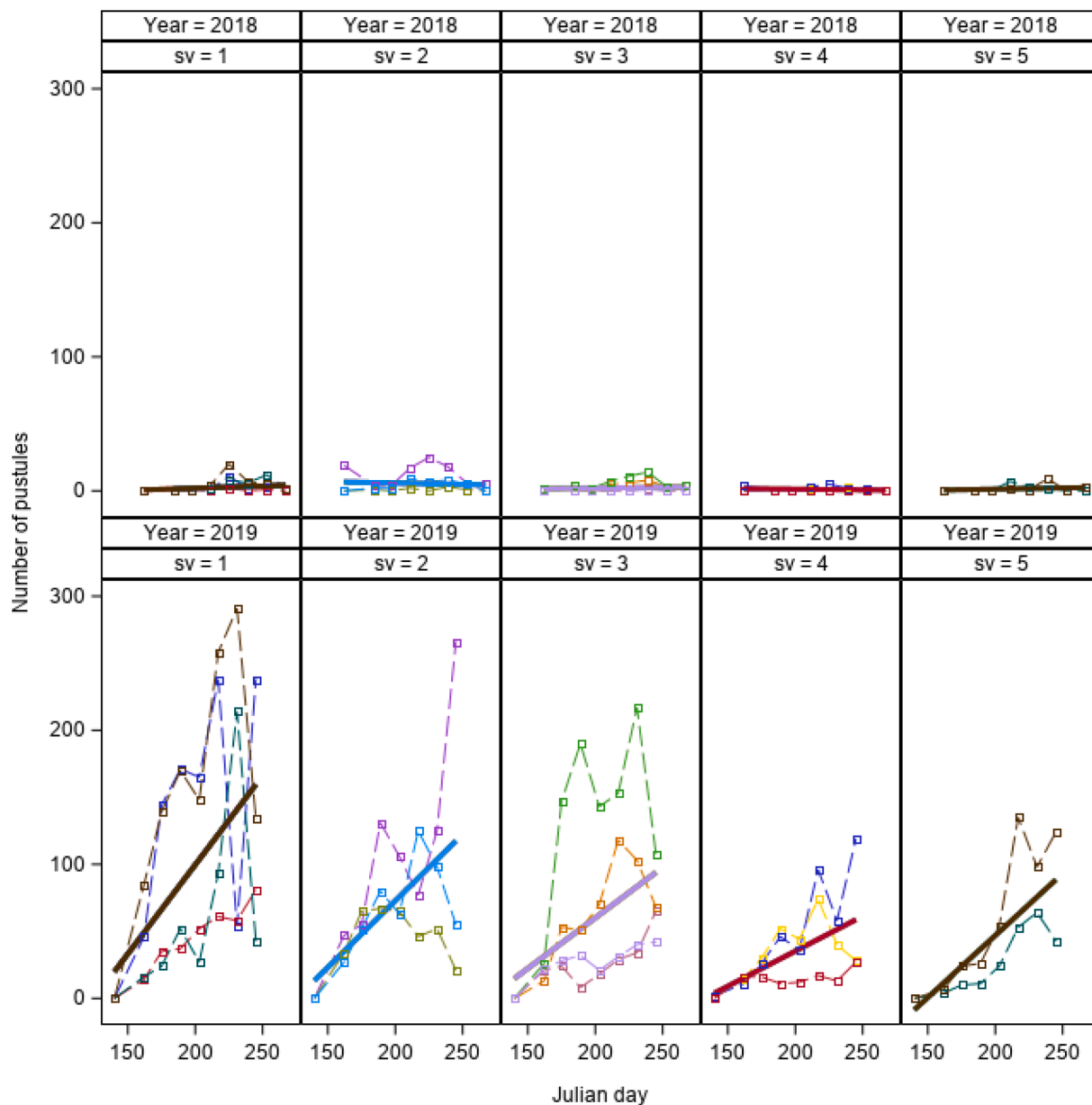


Fig. 7. Model of number of *T. areolata* pustules with uredinia per leaf for trees in seed orchards in 2018-19. The models have been counted separately for different years. SV = Seed orchard. Tree observations are marked with dashed lines and the model prediction with a solid line.

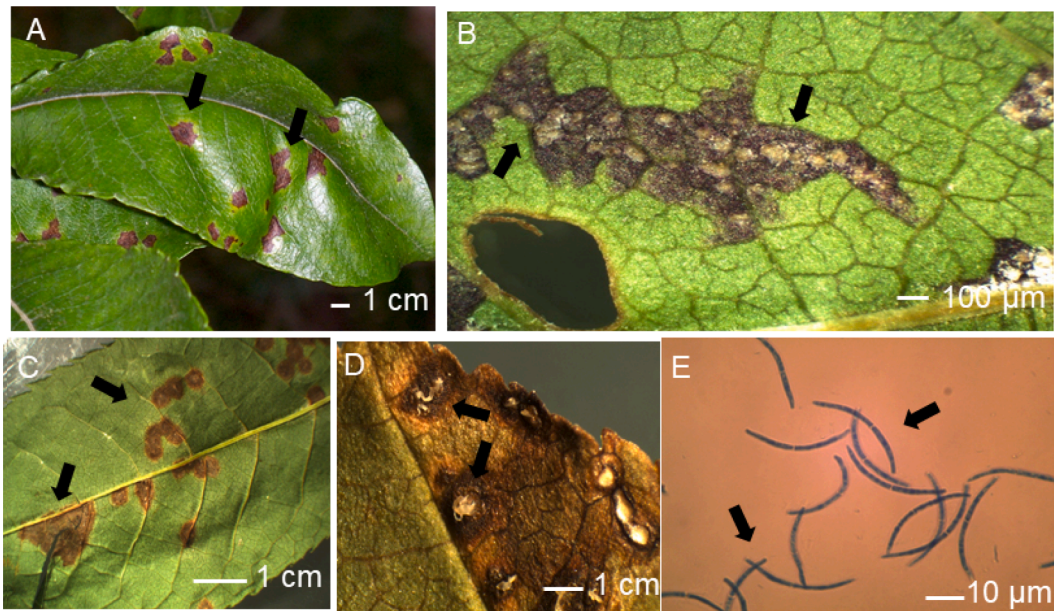


Fig. 8. A. Violet pustules of *T. areolata* with sharp edges (black arrows) and B. yellowish uredinia of *T. areolata* (black arrows) in pustules on lower leaf surface of *Prunus padus*. C. Brownish pustules (black arrows) and D. acervuli of *Phloeosporrella padi* with whitish conidia masses (black arrows) on *Prunus padus*. E. Curved, hyaline, two-celled conidia of *Phloeosporrella padi* (black arrows) stained with lactophenol cotton blue.

uredinia developed on lower leaf surface (Fig. 8b). The violet pustules were usually sharp at the edges and clearly distinguishable from pustules caused by other agents that lacked uredinia. The *T. areolata* pustules were often located close to leaf veins. The pustules turned dark-violet to blackish at the end of the growing season, where telia developed as dark-pigmented layer on the upper leaf surface. We did not confirm teliospore formation in telia, whereas it would have required microtome sample preparation and sectioning for all the sample leaves.

3.3. Temporal variation in coverage of *P. padi* pustules on *Prunus* sample leaves

Pustules caused by *Phloeosporrella padi*, the anamorph of *Blumeriella jaapii*, were observed in all the sample trees both in 2018 and 2019. The coverage of pustules was low in both years, but it increased slightly to <10% until early September. Some variation occurred between trees in the seed orchards both in 2018 and 2019 (Fig. 9). When modelling the variation in average coverage of *P. padi* pustules, seed orchard, time of estimation and interaction between seed orchard and time of estimation were all statistically significant variables in a model, where year and sample tree were random variables (Table 3). The model was:

Coverage of *C. padi* pustules = seed orchard + time of estimation + seed orchard * time of estimation.

Pustules caused by *P. padi* were brownish without sharp edges as a distinguishing feature to violet pustules caused by *T. areolata* (Fig. 8c). The pustules bore acervuli from which whitish masses of conidia protruded (Fig. 8d). The size of acervuli varied from 125 to 450 µm (length; mean = 262 µm, std = 72 µm) to 100–375 µm (width; mean = 190 µm, std = 48 µm). The size of the conidia varied from 32 to 65 µm (length; mean = 50 µm, std = 7,4 µm) to 1,6–3,2 µm (width; mean = 2,5 µm, std = 0,4 µm). The conidiospores were hyaline, narrow, curved with a sharpened tip and a blunt base and contained one septa (Fig. 8e). Based on symptoms and morphology of pustules and conidia, the ascomycete was identified as *Phloeosporrella padi* (Lib.) v. Arx (syn. *Cylindrosporium padi* (Lib.) P. Karst Ex. Sacc.).

3.4. Temporal sporulation of *T. areolata* and *Chrysomyxa* spp. in *Picea abies* cones

The cone rust incidence was low in Norway spruce cones in the seed orchards in 2019. In late May, the cones were still at the pistillate cone stage, but no fruiting stages or disease symptoms were observed in the cones at that time. In June, no spermatogonia or aecia of any of the cone rusts were observed in cone scales (Table 4). Spermatogonia of both *T. areolata* and *C. pirolata* were observed in <1% of the cones in early July. After that aecia of *T. areolata*, *C. pirolata* and *C. ledi* occurred regularly in July-September in 0–7% (*T. areolata*), 0–15% (*C. pirolata*) and 0–9% (*C. ledi*) of the cones per seed orchard (Table 4). Among seed orchards, cones infected by *T. areolata* were most frequent in Sillanpää 235, while cones infected by *C. pirolata* were most frequent in Paronen 365, and cones infected by *C. ledi* in Riihimäki 169 (not shown). In mid-August, cones infected by *T. areolata* and *C. pirolata* could be distinguished from healthy cones by brownish outlook with prematurely opened cone scales (Fig. 10). The cones infected by *C. ledi* remained green similar to healthy cones.

Due to low disease incidence on *Prunus* (Fig. 3), the number of overwintering infected leaves was low in the seed orchards in 2018 that led to low basidiospore dissemination and disease incidence in cones in 2019. Similarly, *Pyrola* sp. was heavily infected by *C. pirolata* in Paronen 365 seed orchard (Table 5) that led to high number of overwintering *Pyrola* leaves in 2018, and further on to high basidiospore dissemination and disease incidence in cones in 2019. The rainy early summer at the time of basidiospore spreading favoured infection of cone rusts to pistillate cones in 2019 (Fig. 4).

3.5. Temporal sporulation of *Chrysomyxa pirolata* on *Pyrola* in a seed orchard

In the beginning of May 2018, undifferentiated fruiting stages of *C. pirolata* developed first on overwintered infected *Pyrola* sp. leaves, when their average coverage per leaf exceeded 100% (Table 5). Until late May, the proportion of leaves with undifferentiated fruiting stages was reduced to 30% with an average coverage of 5% per leaf. After that no undifferentiated fruiting stages were observed. In 2018, first uredinia of *C. pirolata* developed on the overwintered leaves in the first week of

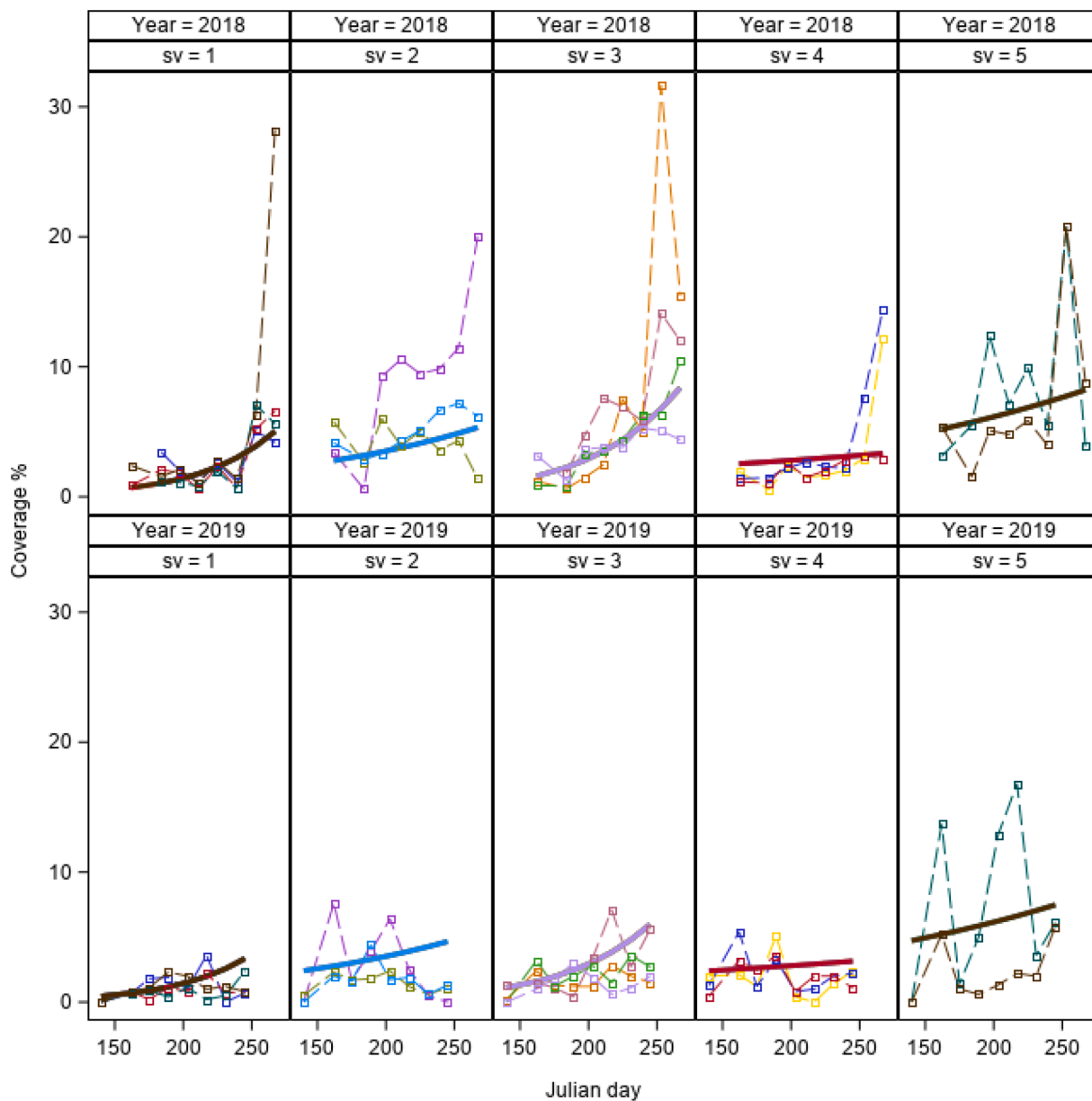


Fig. 9. Model of coverage of *C. padi* pustules with uredinia per leaf for trees in seed orchards in 2018-19. SV = Seed orchard. Tree observations are marked with dashed lines and the model prediction with a solid line.

Table 3

Test of fixed effects (type III) of the general linear mixed model Coverage of *C. padi* pustules = seed orchard + time of estimation + seed orchard * time of estimation in Finnish seed orchards in 2018–19. Random variables are year and tree.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Seed orchard	4	50.31	9.65	<0.0001
Time of estimation	1	124.6	47.44	<0.0001
Seed orchard × time of estimation	4	87.84	7.48	<0.0001

May after which their coverage per leaf and proportion of infected leaves with uredinia increased to 100% at the end of May. Frequency of leaves with uredinia remained high in mid-June, and therefore, the rust sporulated heavily by urediniospores among *Pyrola* in 2018. On the leaves, first telia of *C. pirolata* appeared in late May, but both their average

coverage and proportion of leaves with telia were low until mid-June (Table 5). Therefore, basidiospore dissemination was low in 2018.

In mid-June 2019, the average coverage of uredinia of *C. pirolata* and proportion of leaves with uredinia were much lower than in 2018. The rust spread, therefore, also with urediniospores among *Pyrola* in 2019. However, the proportion of leaves with *C. pirolata* telia with basidia and average coverage of telia with basidia per leaf were much higher in 2019 than in 2018. Therefore, high basidiospore dissemination took place in June 2019. This sporulation was favoured by high rainfall in May-June in 2019 (Fig. 4). The basidiospore dissemination resulted in 15% *C. pirolata* cone frequency in the Paronen 365 seed orchard (Table 4).

4. Discussion

In this study, *T. areolata* incidence was low on *Prunus* trees in 2018 and high in 2019. The frequency of uredinia on *Prunus* leaves revealed that uredinia formation started in late May in 2019 and in mid-June in 2018. Previously, May was reported as the main dissemination time of

Table 4
Frequency (%) of young cones with fruiting stages of cone rusts during growing season in nine Finnish seed orchards in 2019. ¹ = spermogonia, ² = aecia.

Time of estimation	Cones with fruiting stages, %		
	<i>Thekopsora areolata</i>	<i>Chrysomyxa pirolata</i>	<i>Chrysomyxa ledi</i>
20.–22.5.	0	0	0
11.–13.6.	0	0	0
17.6.	0	0	0
24.–27.6.	0	0	0
1.7. ¹	0–1	0–1	0
8.–10.7. ²	0–1	0–1	0–5
18.7. ²	5	2	0
22.–25.7. ²	0–1	0–6	0
5.–7.8. ²	0–5	0–15	0–9
19.–21.8. ²	0–7	0–12	0–8
2.9. ²	6	2	0
2.–5.9. ²	0–4	0–4	0
Max. infected cones/ time	7	15	9

aeciospores in a seed orchard in southern Finland (Kaitera et al., 2009). The aeciospores were disseminated mainly from previous year’s infected cones, and the dissemination took place during the whole growth season (Kaitera et al., 2009). As uredinia are formed 10–14 days after infection on *Prunus* leaves in the laboratory (Kaitera et al., 2019), first uredinia appear on *Prunus* leaves at least two weeks after infection under natural conditions. High rainfall coincided with the time of uredinia formation in the sample leaves in late May to early June 2019, while low rainfall coincided at the same period in 2018, which explained the annual epidemical patterns in the studied seed orchards. It is known that among tree rusts *Chrysomyxa ledi* de Bary (Heino & Pouttu, 2013) and *Melampsora pinitorqua* (Braun) Rostr. (Kurkela, 1973a,b) epidemics are enhanced by high rainfall in early summer. Therefore, also cone rusts *T. areolata* (this study), *C. pirolata* (this study, Kaitera & Tillman-Sutela, 2015) and *C. ledi* (Heino & Pouttu, 2013) are favoured by high rainfall in late spring and early summer. In this study, early infection in May was essential for epidemical development, which lead to 100% disease incidence in 2019 and to lower incidence in 2018. Although weather conditions usually favour disease development in mid- and late summer, the disease patterns increased significantly during the growth season only under high spore pressure but not under low pressure in terms of the number of rust pustules on the leaves. This suggested that spreading of urediniospores was inefficient from tree to tree even under low pressure as seen in slow increase in the *T. areolata* coverage and the

number of pustules per leaf. Inefficient uredinial spreading of the rust during growing season may be due to higher resistance of old leaves compared to young leaves along ageing of the leaves as has been shown in *Cronartium-Vincetoxicum hürundinaria* Medicus pathosystem (Ragazzi, 1986). Therefore, aeciospore infection to young leaves during late May and the environmental conditions at the time have a major effect on epidemical pattern for the whole season in *Prunus*. In the *T. areolata* models, disease incidence, coverage of uredinia and number of uredinial pustules were all insignificantly affected by seed orchard indicating that the same factors affected *T. areolata* epidemical patterns in all Finnish seed orchards both under low and high spore pressure.

Diagnostically the reddish to violet pustules on *Prunus* sample leaves in this study were similar to previous descriptions of *T. areolata* infection after leaf inoculation (Kaitera et al., 2019). The other pathogen on *Prunus* leaves, *P. padi*, however, induced patches with brownish colour that were clearly distinguishable from pustules of *T. areolata* in terms of colour and slimy whitish conidia of *P. padi* (Khan et al., 2016). The coverage of the pustules of the pathogen slightly increased during growth period similar to *T. areolata*, and therefore, rainy periods probably affected disease incidence of the pathogen. In the models, also seed orchard had significant effect on *P. padi* incidence indicating that the pathogen was more common in some of the seed orchards. Diagnostically pustules of these two pathogens could easily be distinguished. The size of acervuli and conidia of *P. padi* observed in this study agree with the descriptions in the literature (Khan et al., 2016). For a seed orchard manager pustules caused the two fungi can be separated by naked eye in terms of presence of uredinia or conidia on the lower leaf surface and colour of pustules.

Table 5
Temporal sporulation of *C. pirolata* on overwintered *Pyrola* sp. leaves in Paronen 365 seed orchard in May-June 2018–19.

Frequency and coverage	Time				
	2018				2019
	7.5.	22.5.	30.5.	11.6.	11.6.
Leaves with fruiting stages (%)					
Undifferentiated fruiting stages	100	27	0	0	0
Uredinia	2	100	100	100	100
Telia with basidia	0	66	13	7	67
Coverage of fruiting stages (%)					
Undifferentiated fruiting stages	99.8	5	0	0	0
Uredinia	0.1	59	97	99	66
Telia with basidia	0	15	4	1	33

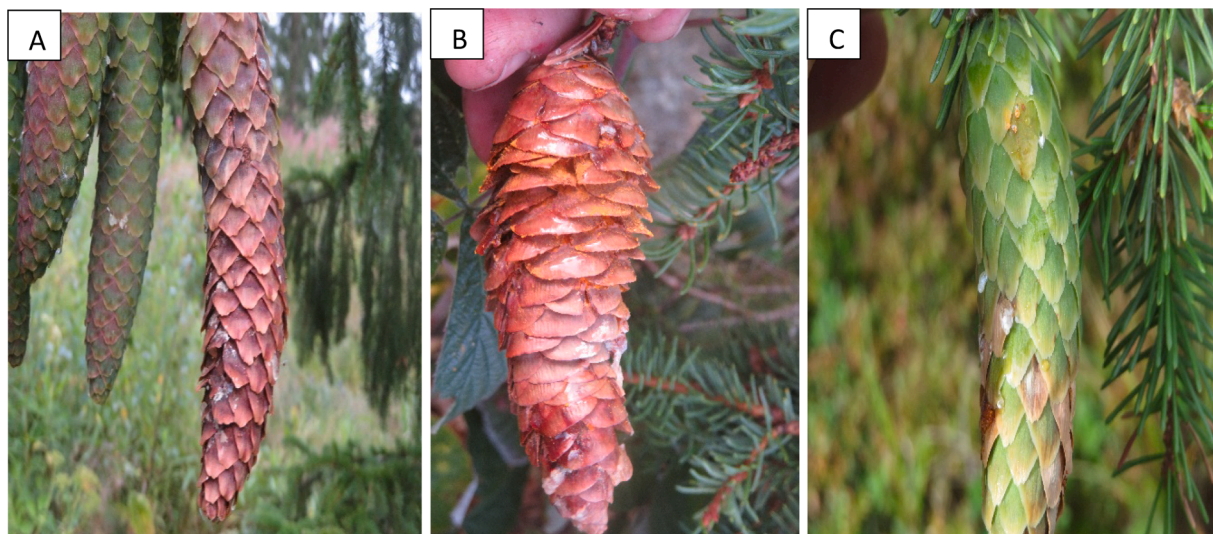


Fig. 10. Young cones infected by A. *T. areolata*, B. *C. pirolata* and C. *C. ledi* with aecia in cone scales in mid-August. A. Healthy cones on the left.

The damage pattern on *Prunus* was clearly affected by the differences in precipitation patterns in the beginning of the growth periods 2018 and 2019. Dry spring in 2018 resulted in low disease incidence of *T. areolata* that lead to a low number of overwintering infected leaves. Therefore, the number of basidiospores was low in the spring 2019 to infect spruce cones in 2019. In this study, this pattern was observed in all the seed orchards in 2019. However, high precipitation in the beginning of the growth period in 2019 enhanced basidiospore spreading of *Chrysomyxa* on alternate hosts, which resulted in high *C. pirolata* and *C. ledi* incidences in cones in some seed orchards where alternate hosts occurred as was shown in this study. The environmental conditions favoured also high aeciospore dissemination and germination on *Prunus* leaves that lead to high disease incidence already in early growing period on *Prunus* in all seed orchards in 2019. Similar epidemical patterns have been shown for *C. pirolata* earlier (Summers et al., 1986; Kaitera & Tillman-Sutela, 2015). Such epidemical waves due to environmental conditions have been reported for *Cronartium* rusts earlier (Peterson, 1971).

In this study, *C. pirolata* sporulated heavily by urediniospores due to low precipitation on *Pyrola* in May–June 2018 resulting in high number of overwintering infected leaves in 2018. The high precipitation in 2019 favoured basidiospore sporulation to pistillate cones in early summer that resulted in high disease incidence in cones as expected and recorded in this study. Precipitation has been shown to be an important factor in telia and basidiospore formation of *C. pirolata* (Crane & Hiratsuka, 2000).

In this study, no fruiting structures of *Thekopsora* or *Chrysomyxa* were observed in cones in June, while spermogonia were observed in early July and aecia in July–August. Earlier, spermogonia of *C. pirolata* and *C. ledi* were observed in *Picea* cones in late June to mid-July and aecia in mid-July to early September (Kaitera et al., 2009, 2010, 2014, 2017), which support our present findings in the seed orchards. Spermogonia of *T. areolata* were also observed in cones in early July and aecia in July–September similar to previous reports (Kaitera et al., 2009, 2014, 2017). At earliest, spermogonia of *T. areolata* have been observed on *P. abies* cones in late June (20th) 2018 (Kaitera, pers. comm.). *T. areolata* and *C. ledi* damage in cones in terms of premature opening of cone scales and browning of cones with aecia formation can in most cases be detected already in mid-August, which is important diagnostically to identify infected cones among healthy ones in seed orchards.

5. Conclusions

In conclusion, as *Prunus* have been shown to be the major susceptible alternate host to *T. areolata*, disease control should be directed to *Prunus* in seed orchards and their close vicinity. Based on distance from the closest *Prunus* to seed orchards, where the disease incidence is moderate to severe, this distance is more than one kilometer. Uredinial spreading from tree to tree is inefficient under low spore pressure in seed orchards, which information is useful in seed orchards. Aeciospores are the most important spores that cause severe rust infection on *Prunus* in early summer depending on weather conditions. Therefore, as infected cones of *T. areolata* sporulate for years after initial fruiting, it is recommended to collect old infected cones from spruce canopy to reduce further sporulation of the rust.

Observing weather conditions and amount of infection on *Prunus* in early summer, the epidemical pattern on *Prunus* can be predicted for the growth period. The number of overwintering infected *Prunus* leaves and the amount of the rust on the leaves give an estimate of the possible amount of dispersing inoculum to pistillate cones in the next spring. In early summer, the weather conditions are crucial for the dissemination.

As *Chrysomyxa pirolata* spreads from overwintered *Pyrola* and *Orthilia* leaves in the spring, control of the rust should be directed to *Pyrola* in early May when the rust is still on the alternate host at the undifferentiated fruiting stage. Later, spraying with fungicides should be directed to pistillate cones in late May to early June depending on the

amount of rainfall before basidiospore production on telia in the *Pyrola* or *Orthilia* leaves.

Infection of *T. areolata* can be identified based on violet pustules on *Prunus* leaves and uredinia formation on lower leaf surface. It is clearly distinguishable from *P. padi* infection with brownish pustules and whitish conidia production on *Prunus*. This information can be used in improving disease diagnostics in seed orchards.

CRedit authorship contribution statement

Juha Kaitera: Conceptualization, Methodology, Writing – original draft, Writing - review & editing, Investigation, Supervision. **Leena Aarnio:** Investigation. **Jouni Karhu:** Data curation, Software, Validation, Visualization. **Tiina Ylioja:** Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kaitera Juha reports financial support was provided by Finnish Ministry of Agriculture and Forestry. Aarnio Leena reports financial support was provided by Finnish Ministry of Agriculture and Forestry. Karhu Jouni reports financial support was provided by Finnish Ministry of Agriculture and Forestry. Ylioja Tiina reports financial support was provided by Finnish Ministry of Agriculture and Forestry. Kaitera Juha reports a relationship with Natural Resources Institute Finland that includes: employment. Leena Aarnio reports a relationship with Natural Resources Institute Finland that includes: employment. Karhu Jouni reports a relationship with Natural Resources Institute Finland that includes: employment. Ylioja Tiina reports a relationship with Natural Resources Institute Finland that includes: employment.

Acknowledgements

This study belonged to MESIKE (<https://www.luke.fi/wp-content/uploads/2018/11/Yleisesittely.pdf>) and SITKE (<https://www.luke.fi/projektit/sitke>) projects financed by the Finnish Ministry of Agriculture and Forestry. We thank Ms. Saija Stranius from Siemen-Forelia Oy for helping in collecting the *Pyrola* leaves, and Mr. Mikko Pulkkinen from Siemen-Forelia Oy and Mr. Hannu Niemelä from Tapio Palvelut Oy for the use of their seed orchards for this study.

Data accessibility statement

Data of this study is available on request from the authors.

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