Cone Bagging Hinders Cone and Rust Development of *Picea Abies*

JUHA KAITERA[1*], EILA TILLMAN-SUTELA[1] AND ANNELI KAUPPI[2]
[1]*Finnish Forest Research Institute, Muhos Research Unit, Kirkkosaarentie 7, FI-91500, Muhos, Finland. E-mail: juha.kaitera@metla.fi Tel.: +358 10 211 3773, Fax: +358 10 211 3701*
[2]*University of Oulu, Department of Biology, P.O. Box 3000, FI-90014 Oulu*

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Abstract

Fruiting and sporulation of Norway spruce cone rusts were investigated in northern Finland. Pistillate flowers and developing cones were bagged at three intervals during the growing season to hinder cone infection by cone rusts, *Thekopsora areolata* and *Chrysomyxa pirolata*. The bagging of cones hindered the maturation and sporulation of *T. areolata* aecia in pistillate cones covered in May, although it also significantly retarded the development of these cones. The bagging fully restricted the formation of *C. pirolata* fruitbodies in cones. The amount of infected cones, however, was very small in general, regardless of the coverage of cones or lack of it during the growing period. In October 40 % of the unbagged cones bore *T. areolata* aecia and 7 % *C. pirolata* aecia. All of the unbagged cones developed normally and the rusts in the infected ones sporulated. Insects were more significant and common than rusts in 2007. The results indicated that rust colonization and fruiting are greatly dependent on the undisturbed development of spruce cones. The total lacking of *T. areolata* spermogonia in the infected cones implies that rust infection process can occur even without basidiospores. The small amount of cone scales bearing fruitbodies of both of the rust species suggests that they hinder the spread of infection of one another in the same cones.

Key words: cherry spruce rust, *Chrysomyxa pirolata*, cone rusts, inland spruce cone rust, Norway spruce, *Thekopsora areolata*

Introduction

Cone rusts frequently cause severe epidemics hitting Norway spruce, *Picea abies* (L.) Karst. in Finland and reducing both the amount and quality of seed crop in seed orchards and natural forests (Kangas 1940, Rummukainen 1960, Nikula and Jalkanen 1990). The role of pathogens accentuates in good cone years (Tillman-Sutela *et al.* 2004) when the amount and quality of collectable seed crop can be reduced essentially. This causes serious problems for northern seed and plant production. The main cone pathogens on Norway spruce in northern Fennoscandia are cherry-spruce rust, *Thekopsora areolata* (Fr.) Magnus, and inland spruce cone rust, *Chrysomyxa pirolata* Wint. (Jørstad 1925, Gäumann 1959, Roll-Hansen 1965). Both species infect pistillate flowers and cones of *Picea* sp. by basidiospores that develop on germinating telia on overwintering leaves of alternate hosts in spring (Gäumann 1959). Sometimes *T. areolata* infects also shoots of *Picea* sp. (Roll-Hansen 1947).

Rainy early summer (May-June) during female florescence of trees and basidiospore dissemination from overwintering leaves of alternate hosts is traditionally considered to be the major factor affecting cone rust epidemics (Summers *et al.* 1985). Other factors affecting rust epidemics are, however, poorly studied and known. As basidiospores are the non-diagnostic spore type in the identification of rusts species, trapping of air-borne spores within the growing season is hardly a useful method in investigating the timing and amount of inoculum reaching the cones. Therefore we chose the method of bagging of developing cones in this pioneer study. Even if rust epidemics have been observed and reported repeatedly, the lack of basic information on factors affecting these epidemics complicates general practice of silviculture and particularly the timing of disease control in seed orchards (Kaitera *et al.* 2007).

To get a more solid basis for rust disease control we studied the phenomenon of cone infection and detailed features of fruiting and sporulation using artificial restriction of inoculum of *T. areolata* and *C. pirolata* to developing Norway spruce cones during the growing season.

Materials and methods

Cone sampling and collection

Norway spruce trees carrying pistillate cones were checked in northern Finland (64° 48'N, 26° 00'E), in early May 2007 to locate trees with sufficient flowering to produce an adequate amount of cones for sampling throughout the growing period. Sample collection was limited to one tree, because it was laborious to collect samples from full-grown trees (h, 24 – 25 m). Only few trees had sufficient flowering in the vicinity of passable roads thus enabling cone sample collection with the help of a lifting cage. In the study area, the cone crop had been excellent in the previous year, in 2006, but simultaneously cone rusts had reduced the seed crop significantly.

Norway spruce branches bearing each ca. 10 pistillate flowers and cones were bagged at three intervals using pollination bags in the upmost part of the canopy of the sample tree. The pollination bags hinder the entrance of small particles to developing cone scales but allow light and moisture to reach the cones. For these reasons bagging is generally used to hinder free pollination in seed orchards. The bagging intervals represented the pollination time of pistillate flowers, the development stage when young cone scales were closed, and the stage when the cones were full-sized but still partly green and soft. The branches carrying cones were bagged in early June (6th June, 9 cones), late June (28th June, 12 cones) and late July (31st July, 13 cones), and the bags were left to envelope cones for the rest of the investigation period. The pollination bags were removed in early October (2nd October), when both the bagged and the rest of the unbagged (15) cones were collected. Half of the cones collected already in June-July were immediately split longitudinally when fresh, and the other halves of the split cones and the whole cones were stored at –20° C. Along the bagging procedure, control cones (a total of 33) were sampled in late May (28-30th May) and at the three bagging intervals, prepared and stored as described above.

Laboratory analysis

All stored cones were checked first when fresh under stereo microscope (Wild M5A). Thereafter, the cones were split into 2-4 longitudinal sections followed by checking each cone scale individually for the occurrence and quantity (%) of cone rust fruitbodies. In pistillate cones, the scales were microscoped for rust spores and hyphae. The occurrences of spermogonia and aecia were recorded. *Thekopsora areolata* aecia are easily distinguishable from aecia of other rusts (Saho and Takahashi 1970). To avoid mixing of *Chrysomyxa pirolata* and *C. ledi* (Alb. & Schw.) de Bary with one another, *Chrysomyxa* spp. aeciospores were identified under light microscope. Species in *C. ledi* complex and *C. pirolata* are very distinct by aeciospore morphology, whereas *C. pirolata* forms a reticulate pattern of warts on the aeciospore surface (Ziller 1974, Sutherland *et al.* 1984, Crane 2001). Spermogonia also differ in morphology between cone rusts (Gäumann 1959, Roll-Hansen 1965, Sutherland *et al.* 1984, Crane 2001). Cross sections of selective samples of rust fruiting stages were made during the first checking. The samples were examined in various orientations using a JEOL JMS 6300F field emission scanning electron microscope (FESEM) to observe detailed features of rust fruitbodies (aecia, aecial peridium) and aeciospores (*e.g.* warts) to confirm the identification of the observed rusts. The ultrastructure of spores and fruitbodies in the sample cones were compared to the findings in the literature. Damage to cones caused by insects was recorded at general level (present/absent) based on descriptions of larvae, adult insects and other feeding symptoms according to Saalas (1949). Disturbance in normal cone development (Owens and Morris 1998) was observed visually at general level (aborted/normal).

Results

Fungal injury was only occasional in the bagged cones. In the cones which were bagged in early June merely pollen was found on cone scales. Meanwhile the regular growth and development of cones had ceased in about 28 % of all investigated cones and in 44 % of the cones bagged in early June. Disturbance in cone development was observed in 33 % of those bagged in late June and in 42 % of the cones bagged in late July.

One cone bagged in early June bore *T. areolata* aecia at the time when the pollination bag was removed in early October, but these aecia were poorly developed, immature and non-sporulating and thus resembling young aecia still in progress. No rust spermogonia were observed in this infected cone. All cones, which were left unbagged throughout the growing season, were fully grown, but they were to some degree affected by cone insects. About 40 % of these unbagged cones carried *T. areolata* aecia and almost 100 % of the scales in these cones were infected in early October, which referred to a systemic infection. Most of these infected cones (83 %) carried no sporulating aecia. Although 17 % of these cones bore sporulating aecia, sporulation was observed only in individual aecia. In addition, almost all (94–98 %) of *T. areolata* aecia appeared on both sides of the scales. Generally aecia were, however, more frequent on the adaxial (inner) than on the abaxial (outer) side of cone scales.

Seven percent of the unbagged cones bore also *C. pirolata* aecia in about 40 % of cone scales in early October, when sporulation had readily finished. On the majority (86 %) of the cone scales, *C. pirolata* aecia occurred on merely the abaxial side of cone scales, even if on 14 % of the scales aecia appeared on both sides of the cone scales. One of the unbagged cones bore both *C. pirolata* and *T. areolata* aecia within the same cone. The two cone rusts occurred together only on 4 % of the cone scales showing that these rusts only rarely colonize the same cone scales. No fruitbodies of *Chrysomyxa* sp. were observed on the bagged or unbagged cones.

Morphologically, the aecia, aecial peridium, and aeciospore surface ornamentation of *T. areolata* and *C. pirolata* developed in unbagged cones were similar to those found recently in a larger study material in Finland (Kaitera unpublished).

In the unbagged control cones which were sampled along the bagging times individual *T. areolata* aeciospores were observed simultaneously with large amounts of spruce pollen on pistillate cones in late May. The control cones collected from May to August had no rust fruitbodies. A few unbagged cones collected in late May, 56 % of the cones collected in late June, and all cones (100 %) collected in late July showed insect damage in seeds and cone scales. In general insects were common damage agents in the sampled cones. *Cydia strobilella* L., *Hylemyia anthraci* Czerny, *Plemeliella abietina* Seitn. and *Euphitecia abietaria* (Goeze) had damaged 100 % of the sampled cones collected in early June, 75 % of those collected in late June, and 58 % of the sampled cones and their seeds collected in late July.

Discussion

In this study, the bagging of cones reduced rust infection and fruitbody development significantly compared to unbagged cones. On the other hand, bagging also impeded normal cone growth and development. The development and maturation of *T. areolata* aecia ceased earlier in the bagged than in the unbagged cones compared to the progress described previously (Gäumann 1959, Saho and Takahashi 1970). Our results imply that aecia of *T. areolata* mature in mid and late summer and consequently are greatly dependent on the normal growth and development of spruce cones. There are no similar studies of the development progress, however, for comparison to confirm these results.

The sporadic and low infection rates of both *T. areolata* and *C. pirolata* in both the bagged and unbagged cones suggest that the incidence of rust inoculum from alternate hosts to cones was small from May to July in 2007. This view is supported by the observed relatively small amount of infected overwintered *Pyrola* spp. leaves carrying *C. pirolata* telia with basidia (Kaitera unpublished). The reduced amount of infected overwintered *Pyrola* spp. leaves coincided with the very poor florescence and cone crop of Norway spruce, which refers to co-evolution between *C. pirolata* and *Picea* spp. This conclusion is also supported by the greater amount of rust diseases in good cone years of *P. abies*, which appear periodically every 6 years in northern forests (Sarvas 1964). Insects were more common than rusts in Norway spruce cones in 2007, which was contrary to the results in the same study area in 2006 when the florescence and cone crop were abundant. Many of the insect species found in spruce cones overwinter in the forest litter and consequently the peak of their population density may be later than that of spruce florescence or cone rusts.

The sporulation of *T. areolata* was frequent in previous year's (2006) cones in early and mid-summer, but no basidia of *T. areolata* were observed on overwintered *P. padus* leaves in the study area in 2007 (Kaitera unpublished). Because the cones which were unbagged throughout the growing period carried higher amounts of *T. areolata* aecia than could be concluded from cones sampled in early-summer, the cones must have received rust inoculum also in mid- and late-summer. An explanation for this could be cone infections by aeciospores from the sporulation in the cones of the previous year, which were adjacent to the sample cones. Norway spruce cones typically stay attached to the branches for two or three years after seed dispersal. Aeciospore infections in mid- and late summer may lead to delayed disease symptoms which do not develop until the end of the growing season. Small amount of infected bagged cones can partly be due to increased temperatures inside the pollination bags, which reduced the normal germination of spores. Even the temperatures above 25°C are known to hinder or reduce significantly the spore germination of some *Cronartium* rusts (Van Arsdel *et al.* 1956, Ragazzi and Mesturino 1986). The total lacking of *T. areolata* spermogonia in the infected cones also implies that rust infection process may occur without basidiospores.

The sporulation of *T. areolata* and *C. pirolata* at the end of the growing season corresponded well to the sporulation of these rusts found previously in Finland (Kaitera *et al.* 2007) and elsewhere (Jørstad 1925, Gäumann 1959, Sutherland *et al.* 1984). The infection and occurrence of both rusts was evidently systemic in cones, which agrees with earlier reports (op. cit.). The small amount of cone scales bearing fruitbodies of both rusts suggests that they hinder the spread of infection of one another in the same cones. Thus rust fungi, which require the living tissue of the host plant, may have responses resembling the allelopathy of higher plants.

Our results support the timing of possible disease control to the period of pollination to prevent the high incidence of fungal infection in the seed orchards. In contrast to the actual practice, the collection and liquidation of contaminated cones could also limit rust infections from old cones and populations of overwintering insects.

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УКРЫВАНИЕ МЕШКАМИ ПРЕПЯТСТВУЕТ РАЗВИТИЮ ШИШЕК И РЖАВЧИННЫХ ГРИБОВ ЕЛИ ОБЫКНОВЕННОЙ *PICEA ABIES*

Ю. Кайтера, Э. Тиллман-Сутела и А. Кауппи

Резюме

Плодоношение и споруляция конидиеносцев ржавчинных грибов шишек исследовались экспериментально на елях обыкновенных в Северной Финляндии. В целях предотвращения инфекции, вызываемой ржавчинами шишек, *Thekopsora areolata* и *Chrysomyxa pirolata*, в определенное время вегетационного периода пестичные цветки укрывались мешками. Укрывание пестичных цветков мешками в мае предотвратило созревание и споруляцию эцидиев ржавчины *T. areolata*, однаю оно также значительно препятствовало развитию шишек. Укрывание мешками также полностью предотвратило развитие эцидиев ржавчины *C. pirolata* в шишках. Однако в целом, количество подверженных инфекции шишек было очень низким независимо от укрывания мешками в вегетационный период. В октябре у 40% неукрытых в течении всего вегетационного периода пестичных цветков наблюдались эцидии ржавчины *T. areolata* и у 7% - эцидии ржавчины *C. pirolata*. Все неукрытые мешкам шишки развивались нормально, а в подверженных инфекции шишках ржавчинные грибы также спорулировали. В 2007 г. насекомые были более значительными и распространенными вредителями, чем ржавчинные грибы. Результаты показали, что вызванный ржавчинами ущерб и их споруляция во многом зависят от ненарушенного развития шишек ели. Полное отсутствие спермациев ржавчины *Thekopsora areolata* в подверженных инфекции шишках подтверждает мнение о том, что подверженность инфекции данным ржавчинным грибом может происходить в отсутствии базидиоспор. Низкое количество чешуек, содержащих эцидии обеих ржавчин, указывает на то, что оба вида ржавчин шишек препятствуют развитию друг друга при распространении инфекции в одних и тех же шишках.

Ключевые слова: ржавчина ели, *Chrysomyxa pirolata*, ржавчины шишек, ржавчина шишек ели, ель обыкновенная, *Thekopsora areolata*