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Colletotrichum acutatum Can Establish on Sweet and Sour Cherry Trees throughout the Growing Season

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Summary

Branches on young (1 to 4 years) trees of sweet cherry 'Lapins' and 'Van' and sour cherry 'Fanal' were artificially inoculated with *Colletotrichum acutatum* at different times during the growing season. Conidial masses of *C. acutatum* were formed on sweet and sour cherry bud scales, flowers, aborted fruits, normally developed fruits, spur leaves, normal sized leaves, and on both one year and older wood pieces when the material was collected two weeks after inoculation, surface sterilized and incubated for two weeks at 20 °C in saturated air. Symptoms on inoculated plant material attached to the trees were only found on normally developed and aborted fruits. Branches of all three cultivars removed one or two years after inoculation developed conidial masses after incubation. Branches of older trees of 'Lapins' and 'Van' were inoculated at full bloom and twice at the green fruit stage and treated like branches on young trees. Conidial masses of *C. acutatum* developed on branches from all inoculation times. Prior to harvest sweet cherry cultivars 'Kristin', 'Lapins', 'Sam', 'Ulster' and 'Van' were inoculated and all expressed fruit symptoms at harvest and after four days at 20 °C. When leaves of sweet cherry cultivars 'Lapins' and 'Van' were inoculated during late summer and autumn, the pathogen produced conidial masses of *C. acutatum* on the leaves incubated both one and two weeks after all inoculations. These investigations show that *C. acutatum* can establish on sweet and sour cherry trees during the whole growing season and that all above-ground parts of the trees may host the pathogen and act as potential sources of inoculum.

Key words. *Prunus avium* – *Colletotrichum acutatum*

Introduction

Anthracnose disease of sour cherry is well-known from different European countries (Grevtseva 1974; Olszak and Piotrowski 1985; Ivanovic and Ivanovic 1992; Kajati et al. 2002; Valiuskaite 2003; Kloutvorovà and Egert 2004; Stensvand and Børve 2010) and once reported from West Virgina in USA (Peet and Taylor 1948). On sweet cherry it has only been reported from Switzerland (Rüegg and Siegfried 1996), Hungary (Holb and Veisz 2005) and Norway (Børve and Stensvand 2006b). *Colletotrichum acutatum* has been reported as the causal agent of anthracnose in sweet cherry in Norway (Stensvand et al. 2006) and Hungary (Magyar and Oros 2012). There is a limited knowledge on when the fungus can establish on sweet or sour cherry trees. Some reports exist on fruit susceptibility to *Colletotrichum* spp. of other stone fruits, e.g. almond and peach. Almond was reported to be susceptible to *C. gloeosporioides* from fruit set to kernel hardening, during six weeks (Shabi and Katan 1983), while peach fruit was susceptible to establishment of latent infections of both *C. gloeosporioides* and *C. acutatum* from two weeks prior to pit hardening and towards harvest (Zaitlin et al. 2000). Investigations of naturally occurring *C. acutatum* have documented that the pathogen can stay asymptomatically on sweet cherry on aborted and normally developed fruits during the green fruit stage (Børve and Stensvand 2004), on bud scales in spring (Børve and Stensvand 2006b) and on leaves throughout the whole growing season (Børve et al. 2010). On sour cherry the fungus has been found on bud scales (Stensvand and Børve 2010), aborted fruits, developed fruits and leaves (Stensvand et al. unpublished data). In experiments in Norway, anthracnose incidence at harvest was strongly reduced by applying fungicides twice at the green fruit stage in both sweet and sour cherry (Børve and Stensvand 2006a). However, time of fruit infections was not determined.

Knowledge about when *C. acutatum* may infect cherry tissue is important for the understanding of the biology of this fungus. This will subsequently help us determine the best timing of control measures to protect the fruits, and

help us minimize the establishment of overwintering inoculum and build-up of an asymptomatic disease pressure during the growing season. Thus, the objective of the present investigation was to find if *C. acutatum* was able to establish on various plant parts of sweet and sour cherry trees at different times during the growing season by artificially inoculating the trees.

Materials and Methods

The major part of the experiments with sweet cherry was done on young, newly established trees with low or no yield. Additionally, older trees with normal yields were used to find if fruits were susceptible at green fruit and prior to harvest. Experiments with sour cherry were done on young trees only.

Young trees

An experimental field of 80 feathered trees each of sweet cherry 'Lapins' and 'Van' and sour cherry 'Fanal' were planted in early spring 2002. Tree spacing was 1.5×3.5 m for 'Lapins' and 'Van' and 1.0×3.5 m for 'Fanal'. Each row had a vegetation free strip of 1 m. No fungicides were applied to the trees, but they were sprayed if needed against insect pests, twice both in 2002 and 2003. The trees were pruned back to having only two branches and topped after planting in 2002. In 2003, they were cut only slightly back, and they were topped at a height of 1.3 ('Van' and 'Fanal') or 1.7 m ('Lapins').

Inoculation

In 2002, an inoculation unit was one branch with 10 buds. The first inoculation of the season in 2002 was done just before bud break, at the swelling bud stage, on one-year old branches with at least 10 buds. Later inoculations also included the new shoot growth emerging from the buds (Fig. 1). In 2003, an inoculation unit consisted of a branch with at least 10 clusters of buds from 2-year-old wood and 10 buds on 1-year old wood. Normally, the first buds located at the base of the 1-year-old wood of sweet cherry are generative while the others are vegetative. On 2-year-old wood of sweet cherry the buds are located in small clusters of generative buds surrounding one vegetative bud (Børve and Stensvand 2006b). Sour cherry branches contains more generative buds on 1-year-old wood than sweet cherry, and the fruit spurs on 2-year-old sour cherry wood are smaller and with fewer buds than on sweet cherry.

The isolate of *C. acutatum* used for artificial inoculations originated from a sour cherry tree in a home garden close to the experimental area. The same isolate was used during all inoculations and was maintained on potato dextrose agar (PDA). To ensure viability of the isolate, repeated inoculations and reisolations took place on apple fruits. Conidial suspensions of 10^5 conidia per ml (adjusted based on haemacytometer counts) were applied to runoff with a hand held spray bottle. Following inoculations, the branches were enclosed in a double layer of milky white plastic bags in order to keep the humidity high, but to avoid large temperature increase during day time, and thus ensure optimal conditions for infection. The bags were removed after two days, and the branches were tagged. Potential germination of the conidia was assessed by placing droplets of suspension on microscope slides kept in saturated air at 20 °C for 24 hours, followed by an assessment of one hundred conidia in a light microscope. A conidium was considered germinated if the germ tube was as long as the width of the conidium. Germination varied from 60 to 100 % over the two years of experiments.



Fig. 1. Conidial masses of *Colletotrichum acutatum* on a sour cherry bud and plant material emerging from the bud.

Collection, incubation and assessment of plant material

Two branches on each of ten trees of each cultivar were inoculated each time, and each branch was inoculated one time during both 2002 and 2003. Inoculations took place six or seven times in 2002 and 2003, respectively (Table 1). Each time of inoculation two branches on each cultivar were treated only with water, serving as controls. One of the two inoculated branches on each tree and one of the water treated control branches were removed 14 days after inoculation, transported separately to the laboratory and then cut into approximately 5 cm long pieces, each containing one bud or the plant material emerging from one bud (spur leaves, new vegetative growth, flowers, fruits, Fig. 1). Each branch piece was surface sterilized by soaking in 0.5 % NaOCl for 1 minute, followed by soaking in 70 % ethanol for 1 minute and rinsing in sterile water for 2 minutes. Then they were air dried on a filter paper and placed separately on a metal

Table 1. Phenological stage and date of inoculation of young trees of sweet cherry 'Lapins' and 'Van' and sour cherry 'Fanal' in 2002 and 2003, and temperature conditions at and just after inoculation.

Year	Phenological stage	Sweet cherry		Sour cherry	
		Date of inoculation	Temperature (°C)[a]	Date of inoculation	Temperature (°C)
2002	Swelling buds	April 24	9.4, 8.5, 7.8	April 24	9.4, 8.5, 7.8
	Full bloom (anthesis)	May 16	9.1, 11.4, 11.8	May 16	9.1, 11.4, 11.8
	Early petal fall	–[b]	–	May 21	14.4, 17.6, 15.1
	Green fruits	June 10	20.0, 15.4, 13.4	June 10	20.0, 15.4, 13.4
	Green fruits	–	–	June 25	12.1, 11.6, 12.1
	Green to yellow fruits	–	–	July 11	15.0, 13.9, 14.8
2003	Bud burst	April 24	10.2, 10.1, 9.3	April 24	10.2, 10.1, 9.3
	20 % open flowers	May 07	8.2, 8.4, 9.6	May 12	9.2, 7.4, 6.6
	Full bloom	May 15	9.3, 8.3, 9.8	May 19	11.0, 11.3, 10.4
	Petal fall	May 22	9.4, 8.8, 10.9	May 28	11.3, 12.0, 12.0
	Green fruits	June 18	15.6, 12.2, 11.1	June 18	15.6, 12.2, 11.1
	Green fruits	July 03	17.4, 19.3, 19.1	July 03	17.4, 19.3, 19.1
	Yellow fruits	July 15	20.2, 21.6, 23.0	July 15	20.2, 21.6, 23.0

[a] Mean daily temperature at day of inoculation and the following two days.
[b] No inoculations of fruits were performed of the young trees of sweet cherry, because they only contained vegetative buds.

grid in a plastic box, with a wet paper towel in the bottom to ensure that the air was water saturated. Two fully developed leaves from each of the branches were detached and then treated and incubated as the branch pieces. The plant parts were checked for visible fungal growth after 7 and 14 days at 20 °C. Total number and incidence of leaves, flowers, fruits, wood pieces, shoot ends (cut ends on vegetative growth from the present year) and buds (the bud itself or material emerging from the bud, Fig. 1) containing sporulating lesions of *C. acutatum* visible in a stereo microscope from each branch were recorded.

The inoculated branches remaining on the trees were assessed for visible symptoms regularly during the growing season. In 2002, no fruits developed on the sweet cherries. At harvest that year, some fruits of sour cherry were picked and incubated for seven days as described above, and incidence of fruits with visible conidial masses of *C. acutatum* was recorded. In total 0 to 18 fruits were harvested and incubated from each of the branches in 2002. September 20 in 2002, ten leaves from each inoculation time were collected, incubated for 3 weeks as described above, and number (incidence) of leaves with sporulating lesions of *C. acutatum* was assessed.

In 2003, five of the ten branches of each cultivar inoculated in 2002, still attached to the trees, were removed just before bud break, treated and incubated using a method described earlier (Børve and Stensvand 2006b). The plant material was not surface sterilized. Vegetative shoots were cut in 5 cm long pieces containing one vegetative bud each, and fruit spurs were detached from the branches. The plant parts were incubated lying separately on metal grids in boxes with wet paper towels underneath the grids to maintain high humidity for two (fruit spurs) or three weeks (shoots) at 20 °C. At time of green fruit, the remaining five branches were removed and incubated as described above for branches inoculated two weeks before incubation. In 2004, four of the ten branches inoculated at time of petal fall and early green fruit in 2003 were removed from the trees and incubated at time of bud break. At that time the branches contained small fruit spurs on the wood that was inoculated the year before and new terminal shoot growth. The new growth was cut in approximately 5 cm pieces with one bud on each, and the fruit spurs were detached from the 2-year-old wood and incubated separately. At bud break in 2005, the remaining branches from the other inoculation times of cv. 'Van' in 2003 were collected, and fruit spurs were incubated as described above.

In 2005, three vegetative shoots (current season growth) of 'Lapins' and 'Van' were artificially inoculated with *C. acutatum* as described above except that the branches were enclosed in milky white plastic bags for three instead of two days. The inoculations were carried out on six occasions at 2-week intervals starting July 1 and ending September 16. One and two weeks after inoculation three leaves with petioles with axillary buds attached from each shoot were removed using a razor blade or a finger nail. The leaves were all from the lower part of the shoot and fully developed. Afterwards the leaves were surface sterilized as described above and then laid on metal grids in sealed plastic boxes, with wet paper towels underneath the grids to maintain high

humidity, frozen at −18 °C for 5 hours, followed by incubation at 25 °C in continuous artificial light and saturated air for 6 days. The incidence of leaves (leaf blade and petiole assessed separately) and corresponding axillary buds with visible sporulation of *C. acutatum* was recorded, and the percentage area of visible sporulation on the blades and petioles (severity) were scored from 0 to 100 at increments of 5.

Older trees in an experimental orchard

In 2002, nine-year-old trees of sweet cherry 'Lapins' and 'Van' (guard trees in a root stock experiment) were inoculated as described above. They were inoculated at time of full bloom (May 10), and twice at green fruit (June 14 and 25), different branches each time. Three branches on each of two trees of 'Lapins' and three branches on each of four trees of 'Van' were inoculated each time. One branch for each inoculation time and cultivar were treated with water and served as controls. The branches were detached two weeks after inoculation, incubated and assessed as described for branches of young trees above. Additionally, ten flowers from each of the branches inoculated at full bloom were collected four days after inoculation and incubated for seven days, being placed separately in incubation boxes as described above, and assessed for visible conidial masses of *C. acutatum*. Also three branches on each of two or three trees of nine year old trees in a root stock experiment of sweet cherry 'Kristin', 'Lapins', 'Sam', 'Ulster' and 'Van' were inoculated as described above, approximately two, three and four weeks (July 3, 11 and 17) prior to anticipated harvest time in 2002. Total number of fruits and number of fruits with visible infections on each branch were counted July 25, five days prior to harvest. Fruits from each branch were collected at normal harvest time, and the healthy fruits were stored at 20 °C for four days. Non-inoculated control fruits were not stored.

Older trees in a commercial orchard

In 2003, ten year old trees of 'Van' in a commercial orchard (Sekse) were inoculated at three different times; full bloom (May 9), petal fall (May 28) and early green fruit (June 17). Each time ten fruit spurs on each of five, four or three branches, respectively, were inoculated as described above. A similar number of branches and fruit spurs were kept as non-inoculated controls (only treated with sterile water). Flowers or fruits from the inoculated branches were collected from each spur separately and incubated for seven days as described earlier. Fruits were collected from inoculated fruit spurs at five different times; the same days as the second and third inoculation (three and six weeks after inoculation), four and two weeks prior to harvest and at normal harvest time. The following year all ten fruit spurs (if still present) from all the inoculated branches were incubated prior to bud break as described earlier (Børve and Stensvand 2006b), but only from 3 control branches (two from June 17 and one from May 9).

Weather data and statistical analysis

Data were obtained from a weather station located at Ullensvang Research Centre, from 0 to 600 m apart from the different research fields and approximately 10 km from the Sekse orchard. Data were statistically analyzed by the GLM procedure of SAS (SAS Institute, Cary, NC), and differences between means were separated by Student Newman Keuls method at $\alpha = 0.05$. Data from each experiment and year were analyzed separately.

Results

Daily mean temperature at day of inoculation and the following two days differed from about 8 to 9 °C at time of swelling buds to above 20 °C in June and July (Table 1).

Young trees

Four weeks after inoculation and incubation (two weeks from inoculation to start of incubation + incubation for two weeks), visible conidial masses of *C. acutatum* were found on one to ten of the ten branches on each of the inoculated cultivars. *C. acutatum* appeared on small spur leaves, normal sized leaves, flowers, normal developed fruits, aborted fruits, wood pieces, shoot pieces and bud scales (Table 2). There was no development of *C. acutatum* on non-inoculated control branches. Incidence per bud (regardless of plant material emerging from the bud, Fig. 1) did not differ with inoculation time for 'Lapins' or 'Van'. For 'Fanal' incidence after inoculation at the yellow fruit stage was significantly higher than at time of swelling buds in 2002 ($P = 0.0006$, Fig. 2). Symptoms on the inoculated branches left on the trees only occurred on aborted fruits or on ripening fruits (data not shown). From 5 to 22 % of incubated fruits of 'Fanal' from the inoculated branches left in the trees developed infections. In the autumn following inoculation in 2002, conidial masses of *C. acutatum* developed on leaves from all branches, independent of inoculation time and cultivar. The incidence varied from 10 to 80 % (data not shown). When buds were collected and incubated at time of swelling buds in 2003, one year after inoculation, conidial masses of *C. acutatum* developed on bud scales from branches of all inoculation times, but not on any of the non-inoculated control branches (Table 3). Also when branches inoculated the year before were incubated at time of green fruit, conidial masses developed after incubation on branches from all inoculation times (Table 3). The conidial masses developed on spur leaves, normal sized leaves, fruits and on the ends of 1-year-old wood pieces. For 'Van' and 'Fanal' there were some significant differences in incidence of *C. acutatum* between the dif-

Table 2. *Colletotrichum acutatum*[a] on plant material from artificially inoculated branches of young trees (1- or 2-year-old) of sweet cherry 'Lapins' and 'Van' and sour cherry 'Fanal'.

Year	Stage of development	Number of infected branches of total inoculated, number of infected buds of total number assessed (type of infected plant material in parentheses)[a]		
		'Lapins'	'Van'	'Fanal'
2002	Swelling buds	4 of 9, 21 of 104 (bud scales)	8 of 10, 14 of 124 (bud scales)	9 of 10, 8 of 173 (spur leaves)
	Full bloom (anthesis)	7 of 9, 11 of 124 (spur leaves)	9 of 10, 9 of 78 (spur leaves)	8 of 9, 20 of 102 (flowers, aborted fruits, fruits, spur leaves)
	Early petal fall	–	–	10 of 10, 22 of 77 (flowers, aborted fruits, fruits, spur leaves)
	Green fruits	10 of 10, 16 of 100 (spur leaf, normal sized leaves)	9 of 10, 21 of 45 (spur leaves, leaves)	9 of 10, 21 of 70 (aborted fruits, fruits, spur leaves, shoots[c])
	Green fruits	–	–	9 of 10, 5 of X[d] (fruits, spur leaves, shoots)
	Green to yellow fruits	–	–	10 of 10, 31 of 71 (fruits, spur leaves, normal sized leaves)
	Controls	0 of 3, no	0 of 3, no	0 of 3, no
2003	Bud burst	2 of 10, 3 of 134 (bud scales)	1 of 10, 1 of 137 (bud scales)	2 of 10, 2 of 155 (bud scales)
	20 % open flowers	8 of 10, 15 of 133 (flowers)	7 of 10, 7 of 130 (flowers, normal sized leaves, wood pieces)	8 of 10, 41 of 154 (flowers, spur leaves, normal sized leaves)
	Full bloom	6 of 10, 10 of 138 (fruits, wood pieces)	1 of 10, 1 of 110 (spur leaf)	5 of 10, 7 of 153, (flowers, spur leaves)
	Petal fall	10 of 10, 120 of 158 (aborted fruits, fruits, normal sized leaves)	10 of 10, 90 of 100 (fruits, spur leaves, normal sized leaves, shoots)	10 of 10, 101 of 149 (aborted fruits, fruits, spur leaves, normal sized leaves, shoots, wood pieces)
	Green fruits	10 of 10, 67 of 229 (fruits, spur leaves, normal sized leaves, shoots)	9 of 10, 65 of 192 (fruits, spur leaves, normal sized leaves, shoots)	10 of 10, X of X (fruits, spur leaves, normal sized leaves, shoots)
	Green fruits	4 of 10, 4 of 115 (fruits, aborted fruits, normal sized leaves)	6 of 10, 1 of 124 (fruits, shoots)	9 of 10, 19 of 74 (fruits, spur leaves, normal sized leaves, shoots)
	Yellow fruits	9 of 10, 25 of 55 (spur leaves, normal sized leaves, shoots)	10 of 10, 24 of 77 (fruits, spur leaves, normal sized leaves)	8 of 10, 16 of 90 (fruits, spur leaves, normal sized leaves)
	Controls	0 of 7, no	0 of 7, no	0 of 7, no

[a] Assessed after incubation for 2 weeks at 20 °C and water saturated air. Incubation started 2 weeks after inoculation (inoculation details, see Table 1) at different phenological stages during 2002 and 2003; 9 or 10 branches were inoculated each time; there were 3 or 7 non-inoculated (treated with water) control branches; all plant material from 10 buds was included from each branch.
[b] Condial masses emerging after incubation were assessed on all plant material originating from a bud; one infected plant part was sufficient to record it as an infected bud. In 2002 only 1-year-old wood was inoculated, while in 2003 both 1-year- and two-year-old wood were inoculated.
[c] Wood pieces were 1- or 2-year-old wood, shoots were current season growth.
[d] Missing data is indicated with X.

ferent inoculation times the previous year when the plant material was incubated at swelling buds, but no differences occurred when incubation took place at the green fruit stage. On 'Van' there was a higher incidence of *C. acutatum* per bud when inoculated June 10 (green fruit) compared to inoculations earlier in the season. On 'Fanal' the incidence per bud was higher on the plant material inoculated at yellow fruit compared to the material inoculated at swelling buds and at full bloom (Table 3). When buds on fruit spurs of 'Van' were incubated in spring 2005 (two years after inoculation), conidial masses of *C. acutatum* developed on 16 to 33 % of the generative buds and on 5 to 13 % of the vegetative buds (each fruit spur normally contains one vegetative bud). No significant differences between inoculation times in 2003 were found (data not shown).

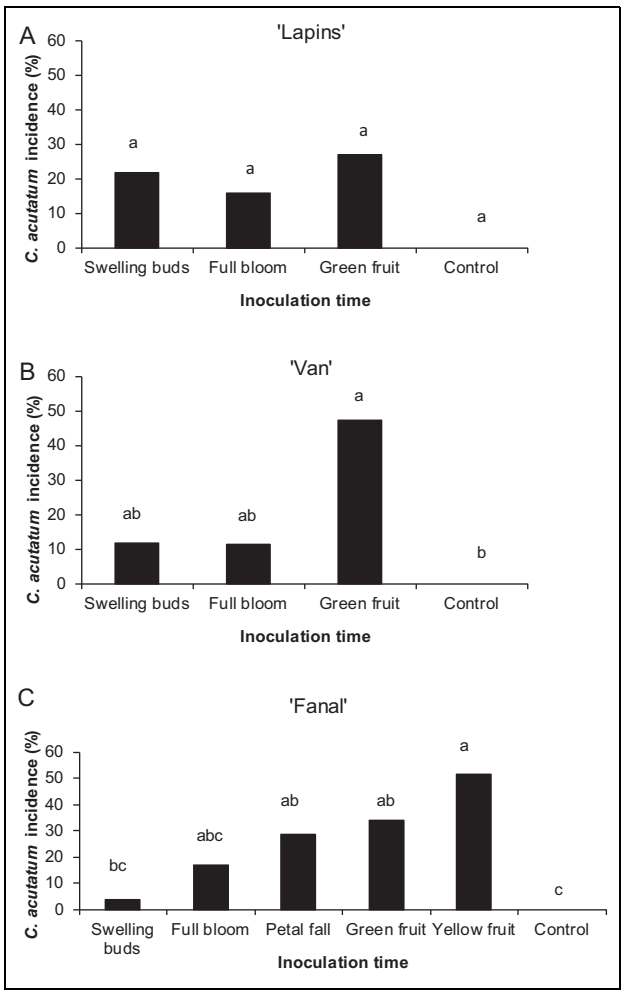


Fig. 2. Incidence of *Colletotrichum acutatum* (%) on buds of sweet cherry 'Lapins' (A) and 'Van' (B) and sour cherry 'Fanal' (C); all plant material emerging from the buds were included. The material was inoculated either at the swelling bud, full bloom, petal fall, green fruit or at yellow fruit stages with *C. acutatum*. Inoculation took place two weeks prior to collection, after which the plant material was incubated for two weeks at 20 °C in water saturated air. Mean of 3 to 10 branches with 10 buds on each in 2002. Mean values with different letters are significantly different according to Student Newman Keuls method at P = 0.05.

Leaves from shoots artificially inoculated from early July to mid-September in 2005 developed conidial masses after incubation, both one and two weeks after inoculation (Table 4). Control leaves also contained the fungus (on 6 to 17 % of the leaf surface), but to a significantly lesser extent than after artificial inoculation. All inoculated leaves developed conidial masses, and the mean percentage of leaf area with visible conidial masses for each inoculation time varied between 50 and 100 %. The highest severity was observed in mid-July and mid-August, the lowest after inoculation in early July (Table 4). Severity of *C. acutatum* was lower (P = 0.0001) on petioles than on leaf laminas (mean of 20 and 61 %, respectively). A mean of 10 % of the axillary buds developed conidial masses after freeze treatments. We did not observe conidial masses on axillary buds from shoots inoculated July 1 and September 2, but if inoculated July 15, August 15, and September 15, respectively, 10, 18, and 2 % developed conidial masses.

Older trees in an experimental orchard

Except from one branch of 'Van' inoculated at full bloom, all branches of 'Lapins' and 'Van' on older trees from the experimental orchard developed conidial masses after all three times of inoculation tested in 2002. On 'Van' there was no significant difference in incidence of fruits with visible sporulating lesions between the different inoculation times (48, 53 and 38 % at May 19, June 14 and June 25, respectively), but on 'Lapins' significantly more fruit symptoms appeared when inoculated June 14 (87 %) than May 10 or June 25 (39 and 32 %, respectively, P = 0.02). Fruits with sporulating lesions were found on non-inoculated control branches after the inoculation June 14, on 22 and 16 % of fruits of 'Lapins' and 'Van', respectively, but not after the other inoculation times. Flowers from inoculated branches developed conidial masses of *C. acutatum* after incubation on 86 of 87 flowers of 'Van' and on 59 of 60 flowers of 'Lapins'. Blighted flowers were not observed on the trees.

On all five cultivars inoculated a few weeks prior to harvest, fruits developed symptoms at harvest (incidence per branch 0 to 28 %) after all three times of inoculation, except 'Kristin' where no symptoms developed on fruits inoculated one week prior to harvest. After storage, symptoms of *C. acutatum* developed on fruits of all cultivars and inoculation times (incidence 12 to 67 %). On 'Ulster', the inoculation closest to harvest had the lowest incidence (1 %) compared to 16 and 14 % when inoculated one or two weeks earlier (P = 0.03, data not shown). For the other cultivars there were no significant differences between inoculation times.

Older trees in a commercial orchard

On older trees of 'Van' inoculated in 2003, the pathogen established on the trees at all inoculation times. *C. acutatum* was found the same year as inoculation occurred, as conidial masses on fruits and flowers, and on bud scales the following spring. The incidence of *C. acutatum* on the buds in April 2004 was from 45 to 87 %, and there was no significant effect of inoculation time or time of collection after inoculation. The pathogen managed to establish on branches from all collection times, and the shortest time from inoculation to fruit collection was 3 weeks.

Table 3. Incidence (%) of *Colletotrichum acutatum* on buds[a] after artificial inoculation of sweet cherry 'Lapins' and 'Van' and sour cherry 'Fanal' in 2002 and assessed the following year.

Inoculation[b] time in 2002	Sweet cherry				Sour cherry	
	'Lapins'		'Van'		'Fanal'	
	Swelling bud	Green fruit	Swelling bud	Green fruit	Swelling bud	Green fruit
April 24	21.5[c]	0.0	11.7 b[d]	4.0	3.8 bc	0.6
May 16	15.9	7.0	11.2 b	0.0	16.9 bc	1.2
May 21	–	–	–	–	28.3 abc	4.1
June 10	26.7	5.5	47.3 a	9.4	33.7 ab	4.6
June 25	–	–	–	–	–[e]	1.6
July 11	–	–	–	–	51.3 a	17.2
Controls[f]	0.0	0.0	0.0	0.0	0.0 c	0.0
P-value	0.36	0.38	0.005	0.34	0.0005	0.09

[a] Buds or the cluster of plant material emerging from the buds were inoculated at different times in 2002, brought into the laboratory and incubated (in saturated air in 20 °C for 2 weeks) at time of the swelling bud or green fruit stages in 2003; if any plant material emerging from one bud was infected with *C. acutatum*, the bud was recorded as infected.
[b] Details on inoculation are found in Table 1.
[c] Mean of 5 branches (replicates) each containing 10 buds for each inoculation time in 2002.
[d] Values denoted with different letters in the same column are significantly different according to Student Newman Keuls test at P = 0.05.
[e] Not assessed.
[f] Five water-inoculated control branches were incubated each time.

Table 4. Percent leaf area (severity) with visible conidial masses of *Colletotrichum acutatum* on sweet cherry leaf laminas of 'Lapins' and 'Van' after artificial inoculations from July to September 2005.

Date of inoculation	Temperature (°C)[a]	'Lapins'		'Van'		Mean
		After one week[b]	After two weeks[b]	After one week	After two weeks	
July 01	18.2, 16.3, 17.0	73.3 b[c]	50.6 bc	64.4 b	58.9 a	61.8 c
July 15	14.4, 15.9, 16.0	90.0 ab	96.1 a	85.6 ab	75.6 a	86.8 a
August 15	14.8, 12.7, 12.7	100.0 a	100.0 a	100.0 a	84.4 a	96.1 a
September 02	15.3, 11.1, 12.4	73.3 b	71.1 b	66.7 b	80.0 a	72.8 b
September 16	8.9, 7.5, 10.9	97.8 a	39.4 b	97.8 a	67.8 a	75.7 b
Controls[d]	–	16.9 c	15.6 c	6.0 c	8.7 b	6.6 d
	P-value	0.0001	0.0001	0.0001	0.0001	0.0001

[a] Mean daily temperature at day of inoculation and the following two days.
[b] Samples were collected one or two weeks after spray inoculation to runoff with 10^5 conidia per ml, and assessed after being frozen at −18 °C for 5 hours and incubated for 6 days at 25 °C in water saturated air and continuous light.
[c] Mean of three replicated samples with three leaves in each. Values denoted with different letters are significantly different according to Student Newman Keuls test at P = 0.05.
[d] Controls are mean of the leaves treated with water from all inoculation times.

Discussion

Infections may establish throughout the season

The present investigation shows that *C. acutatum* can establish on different plant material on both sweet- and sour cherry trees, from time of bud swell in spring until at least one month after harvest. The pathogen had evidently established in the trees because it developed conidial masses two weeks after inoculation on various plant material, in late autumn on leaves, and the following year on bud scales and new plant material developing from

the buds and on woody material. In Norway, overwintering of *C. acutatum* on scales of sweet and sour cherry buds is reported to occur both naturally and after artificial inoculation (Børve and Stensvand 2006b; Stensvand and Børve 2010). Such bud infections are suggested as being a primary inoculum source in spring (Børve and Stensvand 2006b). In the present study we found clear evidence that the fungus could infect at any time and on any leaf, flower, fruit, or green shoot during the growing season. *C. acutatum* on stone fruits (mainly based on investigations in peach), blueberry and apple have been described as having a "fruit hemibiotrophic lifestyle" (Peres et al. 2005), which means that disease symptoms mostly occur on fruits. Observations in the present experiments showed that this is also the case in sweet and sour cherry, and symptoms were only found on aborted and normally developed fruits. After incubation all above-ground plant material produced conidial masses.

Conidial masses on green leaves were sparse when incubated without further treatments than surface sterilization. Freezing sweet cherry leaves prior to incubation has been shown to promote sporulation of *C. acutatum* (Børve et al. 2010), and this was clearly seen in the present experiments in 2005 when leaves were frozen. More sporulation could possibly have occurred if the material was frozen or even treated with paraquat (Cook 1993; Børve et al. 2010) prior to incubation in the first years of investigation. In 2005 it was more sporulation on the leaves at time of sweet cherry ripening (July 15 and August 15) compared to earlier and later. Naturally occurring asymptomatic infections of *C. acutatum* on sweet cherry leaves increased during the growing season and reached a maximum around harvest (Børve et al. 2010).

Mean daily temperature differed from 8 to 23 °C in the course of these experiments. In California, 10 °C was found to be a lower threshold for disease manifestation of anthracnose in almond, although at 10 °C conidial germination of *C. acutatum* was considerably delayed, and a longer wetness period was required for infections to occur (Diéguez-Uribeondo et al. 2004). Experiments with blueberry in Canada indicated that a minimum of 11 hours with leaf wetness at 10 °C were needed to establish fruit infections of *C. acutatum* (Verma et al. 2007). Thus, temperature does not seem to be a limiting factor for *C. acutatum* in the early parts of the cherry growing season in Norway.

The present results indicated a somewhat higher frequency of successful infections by *C. acutatum* on fruits when inoculated at the green fruit stage. Previous experiments in Norway showed that the most important time to apply fungicides against anthracnose on sweet and sour cherry fruits was at the green fruit stage (Børve and Stensvand 2006a). This can be due to more favourable climatic conditions at that time, but also that fruits are relatively susceptible at this stage. Climatic conditions and inoculum availability were the reasons for seasonal peaks in infection of *C. acutatum* on blueberry in Canada, and differences in tissue susceptibility were not important (Verma et al. 2007).

Even close to harvest fruits were susceptible for infection, but not all of the infections became symptomatic before harvest. This indicates that anthracnose can be a potential problem also after storage in heavily infested orchards, and previous experiments in Norway clearly indicates that anthracnose can develop during storage (Børve and Stensvand 2006a).

Earlier investigations in sweet cherry from Switzerland have shown that *C. gloeosporioides* can infect branches and grow in the conducting tissue (Böhni 1949). The present experiments also confirmed that *C. acutatum* may form conidial masses on wood. This has previously been observed for natural infections on sweet cherry in Norway (Børve and Stensvand 2006b). In the present experiments, sporulation occurred mostly on young wood, and this was found both in the year of inoculation and the following year.

Implications for management

Throughout the major part of the growing season, the disease is invisible for the growers. However, an important build-up of inoculum may take place during the time when buds and leaves are formed in summer. The buds will serve as important sources of inoculum the following year, while leaves produce conidia in abundance that may infect the current season fruits. Also old infected fruits overwintering in the trees may serve as inoculum the following spring. Removal of old fruits and proper protection of new growth in spring and summer with fungicides with a high efficacy against anthracnose, may reduce disease pressure substantially.

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