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Interrelationships between Conophthorus Radiatae (Coleoptera: Scolytidae) and Ernobius Punctulatus (Coleoptera: Anobiidae), vectors of the pitch canker fungus in California

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Interrelationships between *Conophthorus radiatae* (Coleoptera: Scolytidae) and *Ernobius punctulatus* (Coleoptera: Anobiidae), vectors of the pitch canker fungus in California

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San Jose State University, 1992

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INTERRELATIONSHIPS BETWEEN *CONOPHTHORUS RADIATAE*
(COLEOPTERA: SCOLYTIDAE) AND *ERNOBIUS PUNCTULATUS*
(COLEOPTERA: ANOBIIDAE),
VECTORS OF THE PITCH CANKER FUNGUS IN CALIFORNIA

A Thesis

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The Faculty of the Department of Biology

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

By

Kelli Hoover

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APPROVED FOR THE DEPARTMENT OF BIOLOGY

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APPROVED FOR THE UNIVERSITY

ABSTRACT

Conophthorus radiatae and *Ernobius punctulatus* infest cones of Monterey pines and appear to be important vectors of pitch canker, a disease of pines caused by a fungus, *Fusarium subglutinans*, which was recently identified in Santa Cruz Co., CA. Where the two beetle species were found together, the percentage of cones containing contaminated *C. radiatae* was greater when *E. punctulatus* progeny were also contaminated (interspecific transmission) than when it was not.

Experimental evidence shows that *C. radiatae* and *E. punctulatus* can transmit the fungus to each other and to their progeny. Either species can transmit the fungus to healthy cones, but *E. punctulatus* required an entrance tunnel by *C. radiatae* to infect the cone.

Interspecific transmission of inoculum between these vectors may enhance the potential for *C. radiatae* and *E. punctulatus* to spread the pathogen to California's native pines. New life history information was obtained for these species.

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INTRODUCTION

This thesis incorporates two closely related manuscripts that were submitted to the entomology journal, *The Canadian Entomologist*, concerning the development of new associations among two insect vectors, *Conophthorus radiatae* and *Ernobius punctulatus*, and the pitch canker fungus, *Fusarium subglutinans*. The first manuscript details the biology and life histories of these insects which led to investigation of their roles as potential vectors. The second manuscript describes the ability of these species of beetles to transmit inoculum of this fungus to each other (interspecific transmission) and to healthy Monterey pines.

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OBSERVATIONS ON THE LIFE HISTORY OF *CONOPHTHORUS*
RADIATAE (COLEOPTERA: SCOLYTIDAE) AND *ERNOBIUS*
PUNCTULATUS (COLEOPTERA: ANOBIIDAE), VECTORS OF THE PITCH
CANKER FUNGUS, *FUSARIUM SUBGLUTINANS*

INTRODUCTION

New life history information for the Monterey pine cone beetle (*Conophthorus radiatae* Hopkins) and *Ernobius punctulatus* Fall was obtained while investigating these insects as potential vectors of the pitch canker fungus (*Fusarium subglutinans* (Wollenw. and Reink) Nelson, Toussoun and Marasas) to Monterey pine (*Pinus radiata* D. Don). Pitch canker disease was discovered in 1986 in Santa Cruz Co., CA (McCain et al. 1987), thus providing opportunities to investigate potential new associations between this pathogen and indigenous insects that infest Monterey pine.

Hepting and Roth (1953) suggested that in the southeastern U.S. insects may serve as vectors of the pitch canker fungus or as wounding agents. In California, Fox et al. (1991) demonstrated that *Ips* spp. transmitted the fungus to Monterey pines. Observations of branch and tip mortality (Adams 1989; McCain et al. 1987), however, suggested that other insects are probably involved in transmitting this pathogen as well.

C. radiatae and *E. punctulatus* are commonly found in Monterey pine in Santa Cruz Co. and are frequently associated with infected tree parts (Fox et al., unpublished). Because *C. radiatae* attacks healthy cones (Ruckes 1958; Schaefer 1962) it was a prime suspect for creating initial infections. *E. punctulatus*, a common associate of *C. radiatae*, (Schaefer, 1962) is polyphagous and may be capable of transmitting inoculum of *F. subglutinans* to *C. radiatae* (i.e. interspecific or horizontal transmission). *C. radiatae* is reported to be host specific (Furniss and Carolin 1977; Wood 1982).

Most *C. radiatae* adults have been observed to overwinter within the cone in which they matured and emerge in February and March to attack second-year Monterey pine cones (Ruckes 1958; Schaefer 1962). In the fall, some of the adults leave the brood cones and attack first-year conelets where they spend the winter (Ruckes 1958). Schaefer (1962) also reported that some newly emerged adults attacked first-year conelets in the first attack phase. Adults of *E. punctulatus* are reported to emerge in the spring and oviposit on or in cones already attacked by *C. radiatae* (Schaefer 1962). Larval development of *E. punctulatus* lags behind that of *C. radiatae* and the two were frequently found in the same cone.

To determine the potential for these beetle species to transmit

inoculum to each other and to Monterey pine, phenological studies of *C. radiatae* and *E. punctulatus* were conducted in 1990-92.

METHODS

Cones that appeared to be infested with *C. radiatae* and/or *E. punctulatus* were collected between June, 1990 and September, 1991 from six sites manifesting varying degrees of pitch canker infection. All sites were located in Santa Cruz Co., CA and contained Monterey pine along with a few bishop pines (*P. muricata* D. Don). The collection sites were described in relation to severity of infection by Fox et al. (1990) as follows: severe infection - New Brighton Beach and Seacliff Beach State Parks; moderate infection - two sites along U.S. Highway #1, intersection at Seacliff Beach Rd. and intersection at Freedom Blvd.; and low infection - Sunset Beach and intersection of U.S. Highway #1 and Mar Monte Ave.

Life history information on *C. radiatae* and *E. punctulatus* was obtained by recording monthly emergence, presence of each life stage in dissected cones, and extent of co-habitation of the two species. Monthly emergence was calculated as the average number of emerged beetles per cone per month. For *C. radiatae* and *E. punctulatus*, the mean time to complete emergence from a single cone and its associated variance were measured as

the mean length of time from first to last emergence from an individual cone. Average number of days to emergence was calculated for *E. punctulatus* to determine the length of its life cycle. Seasonal abundance of each species by life stage in dissected cones was also calculated monthly. The percentage of the population of *C. radiatae* found co-habiting in cones with *E. punctulatus* was calculated for all cones combined to assess the significance of this association in nature.

Ten cones were selected randomly from among the above six sites each week between June and August, 1990. At that time *C. radiatae* had reached adulthood and collections of 10 cones were made twice monthly until October, 1990. Monthly collections of 10 cones were made from October, 1990 through February, 1991 to monitor the overwintering population. In 1991, 10 cones were collected weekly between April and September.

Five cones from each collection were placed in rearing containers (500 ml. ice cream cartons) and five were dissected to determine the number of each life stage that was present. Cones were reared in the Almaden Valley, 33 km. southeast of Santa Cruz, CA. This region has a climate similar to Santa Cruz, which is located on the coast.

RESULTS

Emergence patterns for *C. radiatae* and *E. punctulatus* revealed

unexpected results when compared to prior studies (Ruckes 1958; Schaefer 1962; Hedlin and Strickland 1959; Keen 1958) (Fig. 1). Emergence over the course of the entire life cycle of *C. radiatae* revealed that 48.8% of new adults emerged between June and November while 51.2% emerged from January through March, 1991. However, emergence occurred from 30.6% (15 of 49) of all cones in rearing by the end of September, 1990 compared to emergence from 30% (18 of 60) of all cones between October, 1990 and April, 1991. Peak emergence occurred from July to September and February to March. Emergence of adults in the second year's sample of infested cones (collected April, 1991 through September, 1991) was consistent with the pattern observed in 1990-91 except that minimal emergence occurred after October, 1991. By the end of September, 1991, emergence of *C. radiatae* adults had occurred from 34.6% (34 of 98) of these cones. Between October, 1991 and April, 1991 emergence occurred from only 5.1% (6 of 98) of cones. Therefore, *C. radiatae* new adults emerged from a total of 39.7% of cones June, 1991 through March, 1992 compared to 60.6% of cones June, 1990 through March, 1991. The mean number of *C. radiatae* adults emerged from cones was 5.5 (23) +/- 9.2 for the first year (June, 1990 to April, 1991) compared to 8.0 (34) +/- 9.0 for the second year (emerged June 1991 to April, 1992). The emergence of beetles in summer and early fall coincided with observations of attacks on

first-year conelets. No progeny were produced by *C. radiatae* in conelets. *E. punctulatus* was frequently found together with *C. radiatae* in these first-year conelets.

E. punctulatus adult emergence began in November, 1990, peaked in January, 1991, and was completed by April, 1991. The same pattern began in the second year's sample of cones, but emergence of *E. punctulatus* adults occurred from only 2 of 98 (2%) cones in January, 1992 compared to 14 of 60 cones (23.3%) in January, 1991. The mean number of *E. punctulatus* adults emerged from cones for the first year was 2.5 (25) $\pm$ 3.4 compared to 1.4 (9) $\pm$ 0.7 for the second year.

Half of the reared cones (total = 98) collected April, 1991 through September, 1991 were dissected April 1, 1992 to determine the fate of unemerged beetles. Approximately one-third of this sample (16 of 49) contained one or more parasitized *E. punctulatus* larvae or pupae while 80% (39 of 49) contained dead *C. radiatae* new adults (mean of 4.5 dead adults/cone, SD=5.2, n=49). The exact cause of death was not readily apparent and has yet to be determined. In a few cones cocoons of *Cephalanomia utahensis* Brues (Hymenoptera: Bethyridae) were observed, but the host could not be identified.

In all but two reared cones of the first year's cone collections, the entire brood of *C. radiatae* adults either emerged early or overwintered in the brood cone. The mean time to complete emergence of all brood adults from an individual cone was 37 days (SD=37.4, n=42) with a range of 4-190 days.

E. punctulatus required 6-9 months to complete its life cycle. The average number of days to emergence was 174.1 days (SD=79.2, n=38) with a range of 120-289 days. The time to complete emergence of all brood adults of *E. punctulatus* from an individual cone was 32.5 days (SD=22.5, n=10).

Observations obtained from dissected cones were consistent with results derived from reared cones. The first *C. radiatae* adults were found in dissected cones in mid-June but new brood adult density was highest in August, 1990 and 1991 and January, 1991 (Fig. 2). The first larvae were found in April, 1991, but densities were highest in June of both years. Pupae first appeared in May, 1991 and were most abundant in June.

In dissected cones, the first *E. punctulatus* eggs were observed in April, 1991, larvae in early May and new brood adults in October, 1990 (Fig. 3). On occasion a single adult was recovered from infested cones collected in the summer months, but no pupae were seen until early October. *E. punctulatus* larvae were present all year with the exception of March and April, 1991.

Of those cones that contained beetles of either species, *C. radiatae* and

E. punctulatus were found together in 26.1% (46 of 176) of these cones while *C. radiatae* and *E. punctulatus* were found alone in 53.4% (94 of 176) and 20.5% (36 of 176), respectively. When only *E. punctulatus* was present, there was evidence in 92% (33 of 36) of the observations that *C. radiatae* had been present earlier (i.e. presence of frass, galleries, etc.) but the cone was abandoned without laying eggs or emergence had already occurred. If there was no evidence of *C. radiatae* attack, entrance into the cone had been provided by another insect, usually by a larvae of *Dioryctria auranticella* (Grote) (Lepidoptera: Pyralidae) or *Epinotia hopkinsana* (Kearfott) (Lepidoptera: Olethreutidae).

Up to four larvae of the parasitoid *C. utahensis* were observed on a single late instar larva of *E. punctulatus* in the cones collected and dissected from April, 1991 through September, 1991. Up to 50 adult wasps per cone emerged in the months of July, August, and September, 1991 from 57.1% (56 of 98) of samples collected during this same period. No parasitoids were observed on larvae of *C. radiatae* during either year.

DISCUSSION

Nearly half of the 1990-91 population of *C. radiatae* exhibited emergence from June through November, while the remaining half of the new brood remained within the cone to overwinter. By August all *C. radiatae* had reached the adult stage. Because temperatures at this time still favored emergence, some type of diapause is suggested for the beetles in approximately half of the cones containing overwintering adults. Early emergence correlated with observation of attack by *C. radiatae* adults on first-year conelets.

Early emergence was inferred but not actually observed by Ruckes (1958) and Schaefer (1962). Ruckes (1958) reported the presence of emergence holes in infested cones in August and *C. radiatae* adults in first-year conelets suggesting that there are two overwintering populations, one in the brood cones and one in conelets. However, he did not indicate that cones were dissected to verify the presence of overwintering adults or report the time of year that beetles were observed in these conelets. Schaefer (1962) stated that early emergence occurred in only a small number of cones but he did not indicate the basis for this conclusion.

The cause of nearly 100% mortality in the overwintering *C. radiatae*

population from cones collected April, 1991 through September, 1991 remains unknown. Emergence that did occur between November, 1991 and February, 1992 occurred from only 5 of 98 cones. Miller (1915) reported dead overwintering broods of *Conophthorus lambertianae* Hopkins in over 50% of the cones in an area near Mt. Shasta City, CA in 1913. The cause appeared to be an entomophagous fungus, but no definitive findings were reported.

The lengthy duration of the life cycle of *E. punctulatus* differs from previous reports for *E. punctulatus* and a few closely related species. These differences, however, may be due at least in part to rearing conditions. In this study insects were reared under natural conditions. Hedlin and Strickland (1959) reared *E. punctulatus* from Douglas-fir cones in British Columbia but did not indicate whether the insects were reared under natural or laboratory conditions. Although they observed larvae in Douglas-fir cones from April to September, the overwintering stage was not identified. Larvae were found in Monterey pine cones from April to February. Adult emergence from Monterey pine cones began in November and was completed by April while emergence from Douglas-fir cones began in June. Larvae from Douglas-fir reared in gelatin capsules in the laboratory completed their development in three to four months, but Hedlin and Strickland (1959) did not continue their observations beyond the end of September to determine if larvae can persist

over one or more years. A closely related species, *E. pallitarsis* Fall, was reported by Keen (1958) to emerge over a period of three years. However, there was no indication of larval age at the time cones were collected. *E. melanoventris* Ruckes collected in northern California (Ruckes 1957) overwinter as pupae, but in southern California they emerge as adults in October and produce a second brood. It is possible that the majority of *E. punctulatus* larvae in Monterey pine in Santa Cruz Co., CA enter into some type of diapause during the winter months.

Observations of the parasitoid, *C. utahensis* only on *E. punctulatus* larvae and pupae suggests that although *C. radiatae* larvae were available when adult wasps entered the cones to deposit their eggs, they showed preference for *E. punctulatus* larvae. Ruckes (1956) reported that *C. utahensis* is the principle parasite of *C. radiatae* and Schaefer (1962) indicated that wasps were found on *E. punctulatus* larvae only when *C. radiatae* larvae were no longer available. Schaefer (1962) reported that *C. utahensis* was polyembryonic and produced up to 6 adult wasps per host, although individual wasp size varied inversely with the number of parasitoids produced per host.

According to Schaefer (1962) *C. utahensis* attacked second-instar larvae and pupae of *C. radiatae* in May and June. Adult wasps overwintered in the

brood cones with *C. radiatae* adults and were observed to emerge the following May and June, after *C. radiatae* emergence although some fall emergence occurred. It was suggested that fall emergence occurred principally for dispersal. In the present study *C. utahensis* adult emergence occurred in high numbers in July, August, and September and no live adult wasps overwintered in the brood cones.

C. radiatae and *E. punctulatus* were found together in about 25% of the observations indicating the potential for interspecific transmission of inoculum of *F. subglutinans* ,thereby enhancing their potential vectoring capability. Emergence of large numbers of the parasitoid, *C. utahensis* represents another potential source of inoculum. Wasps may become contaminated with propagules from parasitized late instar larvae of either beetle species and either directly inoculate a healthy *C. radiatae*-infested cone and/or introduce propagules that can be acquired by unparasitized beetle larvae of either species.

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Fig. 1. Mean number of adult *C. radiatae* and *E. punctulatus* emerged per cone per month from cones collected from June, 1990 through September, 1991. Averages were computed only from cones exhibiting emergence. (n=60 June, 1990 through April, 1991, n=98 May, 1991 through March, 1992)

Fig. 2. Mean number of progeny of *C. radiatae* per cone by life stage dissected from cones collected June, 1990 through September, 1991. (n=116 June, 1990 through March, 1991, n=98 April, 1991 through September, 1991)

Fig. 3. Mean number of progeny of *E. punctulatus* per cone by life stage dissected from cones collected June, 1990 through September, 1991. (n=116 June, 1990 through March, 1991, n=98 April, 1991 through September, 1991)

TRANSMISSION OF THE PITCH CANKER FUNGUS, *FUSARIUM*
SUBGLUTINANS, BETWEEN THE VECTORS *CONOPHTHORUS*
RADIATAE (COLEOPTERA: SCOLYTIDAE) AND *ERNOBIUS*
PUNCTULATUS (COLEOPTERA: ANOBIIDAE)

INTRODUCTION

Recent discovery of pitch canker, a disease of pines caused by the fungus, *Fusarium subglutinans* (Wollenw. and Reink) Nelson, Toussoun and Marasas, on Monterey pines (*Pinus radiata* D. Don) in Santa Cruz Co., CA (McCain, et al. 1987) offered unique opportunities to investigate the development of new associations between this pathogen and potential insect vectors. A large number of pine species are susceptible to this disease, but the strain(s) of *F. subglutinans* present in California are especially pathogenic to Monterey pine (McCain et al. 1987). Disease symptoms present on Monterey pine in Santa Cruz Co., CA include resin streaming, branch and bole cankers, shoot die-back, cone death, and in some cases, tree mortality. Inoculation studies on Monterey pine in California resulted in stem girdling in 3 weeks or less (McCain et al. 1987). Since it was first identified in Santa Cruz Co., pitch canker has been found in nine other counties in California infecting several other planted pine species (Adams 1989).

Conditions favoring spread of pitch canker appear to differ between the southeastern U.S. and California. In the southeastern U.S., infection through airborne or rain splashed spores into mechanically created wounds result in multiple infections throughout the tree (Barrows-Broadbent 1987; Blakeslee et al. 1981). In California infection by airborne inoculum through mechanical or weather-related injuries does not appear to be an important mechanism in the spread of the disease (Correll et al. 1991). In California, Fox et al. (1991) demonstrated that the California five-spined ips, *Ips paraconfusus* Lanier, transmitted the fungus to living pine hosts when experimentally-contaminated and confined on the host. Pheromone-induced attacks by *I. paraconfusus* and *Ips mexicanus* Hopkins resulted in cankers from naturally-contaminated beetles.

Patterns of disease spread, however, suggested that other insect species were probably involved in the transmission of this fungus to Monterey pine. *Conophthorus radiatae* Hopkins, *Ernobius punctulatus* Fall, and *Pityophthorus carmeli* Swaine¹ were implicated as potential vectors because of their biology. Each of these species has been shown to carry *F. subglutinans* propagules at fairly high levels (>10%) and have been found in

¹ *Pityophthorus carmeli* will be referred to as *Pityophthorus* spp. as recent expert identification revealed a mixture of *P. carmeli*, *P. nitidulus* (Mannerheim), and *P. setosus* (Blackman) in an approximate ratio of 4:2:1 in samples collected from Monterey pines at these sites.

pitch canker-infected tree parts (Fox, et al. unpublished). Because *C. radiatae* attacks healthy cones, it was a prime suspect for creating initial infections. Nearly 50% of new infections occurred at cone whorls in some stands in Santa Cruz Co., CA (Fox et al., unpublished) and *Pityophthorus* spp. are known to attack declining twigs, and were unexpectedly found in Monterey pine cones in this study.

In addition, *C. radiatae* may be capable of interspecific transmission of inoculum with its associate, *E. punctulatus*. *C. radiatae* was found with *E. punctulatus* in 26% of cone samples, but evidence suggested that co-habitation may be closer to 50% (Hoover et al. 1992). The life histories of *C. radiatae* and *E. punctulatus* are summarized in Fig. 1 and detailed phenological data have recently been reported (Hoover et al. 1992). *C. radiatae* is monophagous on Monterey pine but *E. punctulatus* is polyphagous (Furniss and Carolin 1977). If these insects are capable of interspecific transmission of inoculum, the spread of pitch canker may be increased substantially.

In order to determine if these species were vectors of this pathogen it was necessary to satisfy Leach's principles of insect transmission of plant diseases (1940). Investigations were made to address the following: 1) the extent of contamination of each species with *F. subglutinans*; 2) the extent of

association between contaminated beetles and the presence of the fungus in cones; 3) the transmission of the fungus to beetle progeny; and 4) transmission of the fungus to healthy Monterey pine cones.

METHODS

Six sites located in Santa Cruz Co., CA manifesting varying amounts of pitch canker disease were used in this study (Hoover et al. 1992). Two sites in each of the following categories were included: a preponderance of trees with none to low, moderate or severe infections as characterized by Fox et al. (1990). All sites contained Monterey pine along with a few bishop pines (*P. muricata* D. Don). Using standard sterile technique, the presence of *F. subglutinans* was determined by plating samples on a medium selective for the pitch canker fungus (McCain, unpublished).

Natural contamination of *Conophthorus radiatae*, *Ernobius punctulatus*, and *Pityophthorus* spp. captured in flight. The percentages of *C. radiatae*, *Pityophthorus* spp., and *E. punctulatus* naturally contaminated with *F. subglutinans*' propagules were examined at the above six sites. However, in January, insects trapped at Sunset Beach were found carrying spores of *F. subglutinans*. An additional site was added in Monterey Co., CA that was

considered a none to low pitch canker infection site, located 1.6-km South of the Pajaro River in a small stand of Monterey pines for trapping only.

Monthly trapping of target species began in June, 1990 and was continued through June, 1991. Sticky traps were prepared from plastic screen cut into pieces 25 cm by 10 cm and stapled around a developing conelet group (1 yr old). The screen was sprayed with commercially available Tangle Trap® and left in place for 2 wks. A maximum of 100 trapped individuals were used to determine the monthly percentage contamination of each beetle species by this fungus. Because *Pityophthorus* spp. were found in infested cones and conelets during this study, this species was included in the trapping studies.

Acquisition of *Fusarium subglutinans* by beetle progeny under natural conditions. The extent of natural contamination of beetle progeny was examined from June, 1990 through September, 1991 by randomly selecting 10 cones from each of the six sites in Santa Cruz Co., CA for collection each week through August, 1990. At that time *C. radiatae* had reached adulthood and collections of 10 cones were made twice monthly until October, 1990.

Monthly collections of 10 cones were made from October, 1990 through February, 1991 to monitor the overwintering population. Data for a second year was obtained by randomly selecting 10 cones for collection every 2 weeks from April, 1991 through September, 1991. Half of the cones from each

collection were placed in rearing containers in the Almaden Valley, 33 km. southeast of Santa Cruz, CA. This region has a climate similar to Santa Cruz, which is located on the coast. The remaining half of each cone sample was dissected at the time of collection to ensure that the experimental conditions for rearing did not alter the timing of beetle emergence or levels of contamination. All insects from each cone were plated on a selective medium (McCain, unpublished).

Contamination levels for each species and life stage were calculated by two methods. For each beetle species, the percentages of the total population carrying inoculum of *F. subglutinans* from reared and dissected cones combined was calculated as the total number of contaminated individuals divided by the total number of collected individuals. Because it was unknown whether the disease has a contagious distribution, it was also important to measure the percentage of individuals contaminated per cone and the associated variance. Mean percentage of contaminated individuals per cone was calculated as the number of contaminated individuals in a cone divided by the total number of cones. Reared and dissected cones were computed separately. Pearson's Chi-square test (Zar, 1984) was used to determine if mean percentage contamination varied between reared and dissected cones.

Interspecific transmission between *C. radiatae* and *E. punctulatus* under natural conditions. To examine the potential for interspecific transmission of inoculum between *C. radiatae* and *E. punctulatus*, a Pearson's Chi-square test (Zar, 1984) was used to test the association of contaminated *C. radiatae* and the presence of contaminated *E. punctulatus*. The same test was used to examine the association of contaminated *C. radiatae* and the presence of *E. punctulatus* regardless of *E. punctulatus* contamination status.

Transmission of *F. subglutinans* to healthy Monterey pine cones and conelets under natural conditions. The association of *C. radiatae* and *E. punctulatus* with infected cones under natural conditions was determined by tagging 80 beetle infested cones (determined by the presence of a pitch tube and/or lack of normal cone development) distributed among the six sites in Santa Cruz Co., CA in August, 1990. Monthly examinations for symptoms of pitch canker (canker development at the base of the cone) were made through May, 1991. Those cones that displayed symptoms of pitch canker were collected and dissected. Resident insects were plated on selective medium. The association between pitch canker symptoms and the presence of contaminated beetles was investigated by determining the proportion of cones that developed pitch canker by infesting species, mean number of beetles recovered per cone, and mean percentage contamination of each species per

diseased cone.

Early in the study it was observed that first-year conelets at the six Santa Cruz Co., CA sites were frequently being attacked by *C. radiatae* and/or *Pityophthorus* spp. adults. To determine the pattern of attack and/or infestation and possible pitch canker infection of these conelets, 25 apparently healthy conelets were tagged at each Santa Cruz Co., CA site in June, 1990 and examined monthly through May, 1991 for evidence of beetle attack and/or pitch canker infection. Conelets that displayed signs of attack or discoloration were collected and dissected. Resident insects were identified and plated on selective medium. An attempt was made to isolate *F. subglutinans* from conelets that appeared to have symptoms of pitch canker (i.e. resinosus and yellow-brownish discolored tissue) whether beetles were present or absent.

The proportion of conelets attacked by species per month summed over all areas and mean percentage of resident insects contaminated were calculated. Pearson's Chi-Square test (Zar, 1984) was used to compare the proportion of contaminated insects with the proportion of conelets positive for *F. subglutinans*. Average progeny of each species per conelet and frequency of association of *C. radiatae* and *E. punctulatus* with each other were also determined.

Because it was unknown whether symptoms of early insect attack and/or development of pitch canker would be obvious from visual examination of developing conelets, a random sample of 25 conelets was collected from the six sites in Santa Cruz Co., CA monthly from June, 1990 through January, 1991 and dissected. Attacking adults and their progeny were identified and plated on selective medium. The number of attacks by species each month, mean percent contamination of resident insects, mean progeny per cone, and the proportion of beetles found in various associations were calculated.

Transmission of *F. subglutinans* to Monterey pine cones under experimental conditions. In February and March, 1991 an experiment was conducted to determine whether *C. radiatae* and *E. punctulatus* have the capacity to transmit *F. subglutinans* to healthy cones and, if so, which contaminated insect or combination of insects are most effective as vectors. Twenty replicates of the 9 groups outlined in Table 1 were established at 2 sites where second-year developing conelets were abundant and pitch canker was not well established (Sunset and Freedom Blvd.). *E. punctulatus* was not introduced to a cone first followed by *C. radiatae* because it was determined that *E. punctulatus* was unable to obtain entrance to a cone without an entrance tunnel provided by another insect (Hoover et al. 1992).

One insect of each species was confined per cone whorl using plastic screen tied with twine to prevent immigration or emigration. *C. radiatae* used in the study were reared from cones collected from all sites and were caged as they emerged. *E. punctulatus* were collected by beating foliage at Sunset Beach and Freedom Blvd. A portion of the population of each species used in this study as controls were plated on selective medium to determine the frequency of natural contamination. Beetles to be artificially contaminated were sprayed to run-off with a *F. subglutinans* (JF 139)² spore suspension of 5×10^3 conidia/ml. Where *E. punctulatus* was caged with *C. radiatae*, *E. punctulatus* was introduced one week after *C. radiatae* was confined (Table 1).

All samples were collected in late June and early July, 1991 and examined for evidence of insect attack or pitch canker infection. Attacked cones were dissected and all progeny plated on selective medium. An attempt was made to isolate *F. subglutinans* from all cones.

A hierarchical log-linear model (Zar, 1984) was used to determine if the incidence of pitch canker was related to the number of attacked cones and if there were any differences in disease incidence between experimental groups of artificially contaminated beetle species (Table 1). The same model was

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used to compare the incidence of pitch canker when contaminated progeny were present.

Intra- and interspecific transmission between *C. radiatae* and *E. punctulatus* under experimental conditions. Data from the above experiment was tested using a hierarchical log-linear model to investigate the occurrence of intra- (vertical) and interspecific (horizontal) transmission of inoculum.

Intraspecific transmission was examined by comparing the frequency of cones that contained contaminated progeny when a contaminated parent was introduced, against the frequency of cones that contained contaminated progeny when an uncontaminated parent was introduced. Interspecific transmission was investigated via the same model by comparing the proportion of cones that contained contaminated *C. radiatae* progeny (uncontaminated adult) when an artiificially contaminated *E. punctulatus* adult was confined. The same test was used to determine interspecific transmission to *E. punctulatus* progeny from a contaminated *C. radiatae* parent (again, the introduced *E. punctulatus* parent was uncontaminated for this test). Mean progeny per cone and mean contamination of these progeny per cone were calculated for all treatment groups combined. Mean contamination per cone was also calculated for each treatment group separately by dividing the number of contaminated progeny by the number of

cones in each group for each species and life stage.

RESULTS

Natural contamination of *Conophthorus radiatae*, *Ernobius punctulatus*, and *Pityophthorus* spp. captured in flight. When calculated over all observations from June, 1990 through June, 1991, the percent contamination of *Pityophthorus* spp., *E. punctulatus*, *C. radiatae*, and debris with *F. subglutinans* captured in sticky traps was 13.6%, 11.6%, 33.3% and 1.2%, respectively. The trend in percent contamination of *Pityophthorus* spp. caught in sticky traps varied from 0-43.0% (Fig. 2). Inoculum level on this species was highest in September, followed by January at 25.0%. *E. punctulatus* contamination peaked in June (44.7%) and September (40%) and was lowest in August and November (0%).

E. punctulatus adults were trapped in twice the number (575) compared to *Pityophthorus* spp. (238) (Fig. 3). Sixty-five percent of all trapped *E. punctulatus* were caught from February through April, while *Pityophthorus* spp. were most frequently trapped June through October, with a second peak in February. Only 1 *C. radiatae* was trapped in October, November and February and only one of these was contaminated with *F.*

subglutinans (Figs. 2 and 3).

Acquisition of *Fusarium subglutinans* by beetle progeny under natural conditions. Percent contamination of the total population for each species from reared and dissected cones combined showed that 21% of *C. radiatae* adults, 10.8% of pupae and 8.5% of larvae carried propagules of *F.*

subglutinans. Percent contamination of *E. punctulatus* adults and larvae were 25.3% and 26.9%, respectively. The mean numbers of *C. radiatae* adults, larvae, and pupae per cone were 6.8 (SD=7.9, n=73, range=1-32), 12.1 (SD=13.7, n=37, range=1-65), and 4.3 (SD=4.6, n=23, range=1-40), respectively. The mean numbers of *E. punctulatus* adults and larvae was 3.8 (SD=4.3, n=4, range=1-10) and 3.1 (SD=3.2, n= 57, range=1-13), respectively.

Mean contamination of *C. radiatae* adults per cone from reared cones was 24.7% (SD=0.3, n=24) and 29.5% (SD=0.4, n=26) for *E. punctulatus*. These figures were not significantly different from those obtained for dissected cones. The mean number of emerged *C. radiatae* adults per cone during the year was 4.4 (SD=9.2, n=23, range=1-40) and 1.9 (SD=3.4, n=25, range=1-15) for *E. punctulatus*.

Thirteen dissected infested cones collected in 1990-91 yielded an average of 3.9 *Pityophthorus* spp. adults per cone (SD=4.0, n=13) and an

average of 2 *Pityophthorus* spp. larvae per cone (SD=1.4, n=2). None of these beetles was contaminated. The majority were found between May and July. Only 2 adults were found in dissected cones in January.

Interspecific transmission between *C. radiatae* and *E. punctulatus* under natural conditions. When *C. radiatae* and *E. punctulatus* co-occurred, the proportion of contaminated *C. radiatae* adults was significantly greater (94.0%) when *E. punctulatus* was contaminated than when it was not (35.6%) (Fig. 4). The same significant relationship was found if data from reared and dissected cones were analyzed separately. If the contamination status of *E. punctulatus* is ignored, the frequency of cones containing contaminated *C. radiatae* was independent of the presence or absence of *E. punctulatus* (p=0.11).

Transmission of *F. subglutinans* to healthy Monterey pine cones under natural conditions. Ten percent of tagged *C. radiatae*-infested cones developed pitch canker (10% of all cones, 33% of all whorls). The number of cones exhibiting pitch canker symptoms was highest in January, 1991, and lowest in November, December, February, and April, 1991 (Fig. 5). Of the tagged attacked cones that developed pitch canker, 51.9% (14 of 27) were infested by *C. radiatae* and 11.6% (3 of 27) by *Pityophthorus* spp. Of the cones attacked by *C. radiatae* and *Pityophthorus* spp. that displayed symptoms of

pitch canker, 63% (17 of 17) developed an obvious canker at the cone whorl.

Mean contamination of resident insects was 100% for *C. radiatae* (SD=0), 41.7% for *Pityophthorus* spp. (SD=50.0%) and 40% for *E. punctulatus* (SD=54.8%). An average of 1.0 *C. radiatae* adults (SD=0.4, n=7), 6.3 *Pityophthorus* spp. adults (SD=9.8, n=4), and 1.0 *E. punctulatus* larva (SD=0, n=4) were recovered per cone collected that contained live beetles. One *Ernobius* larva was found alone, 2 with *Pityophthorus* spp. adults and 1 with a *C. radiatae* adult. The *E. punctulatus* larvae found with *Pityophthorus* spp. adults were both carrying inoculum. One contaminated *C. radiatae* was found with an uncontaminated *Pityophthorus* spp. adult.

Twenty-eight percent of 460 tagged observational conelets were infested by at least one of the study beetles and 5% of tagged conelets were collected for reasons discussed later. *C. radiatae* attacks peaked in January and it consistently attacked a larger number of conelets each month than *Pityophthorus* spp. (Fig. 6). Seventy percent of infested conelets were attacked by *C. radiatae*, 29% by *Pityophthorus* spp. and 1% by a lepidopteran larva.

Of those conelets that were killed by other causes, 14 exhibited shoot die-back (1 had pitch canker); 3 were attacked by *Vespamina sequoiae* (Hy.

Edwards) (none had pitch canker); 4 developed pitch canker at the primary cone whorl (1 conelet had pitch canker); and 4 had a branch canker resulting in dead conelets (1 conelet had pitch canker). Branches with shoot die-back resulting in conelet death were consistently mass attacked by *Pityophthorus* spp. and it was not possible to determine whether the conelet was inoculated directly or was infected by spread of the fungus from the shoot.

Twenty-nine percent of infested conelets were positive for *F. subglutinans*. The proportion of these conelets exhibiting pitch canker symptoms and containing contaminated beetles (95-100%) was significantly different from the proportion of symptomatic conelets containing beetles without the fungus (0-26%) (Fig. 7, $p=0.02$ for *C. radiatae*, 0.00 for *E. punctulatus* larvae, and 0.00 for *Pityophthorus* spp adults and larvae). Progeny were produced in conelets by *Pityophthorus* spp. and *E. punctulatus*, but not by *C. radiatae* (Table 2). *E. punctulatus* larvae were present in 15.5% of *C. radiatae*-infested conelets ($n=91$) and 16.1% of *Pityophthorus* spp.-infested conelets ($n=35$). *Pityophthorus* spp. larvae were present in 22.5% of *Pityophthorus* spp.-attacked conelets. Average percent contamination per conelet was highest for *Pityophthorus* spp. adults (40.7%), followed by *C. radiatae* adults (33.3%) (Table 2).

A male/female pair of *I. mexicanus* (Hopkins) was found at the base of

a cone whorl infected with pitch canker. Both were carrying inoculum and the branch had been mass attacked by *Pityophthorus* spp. Twenty-one adults were found in the cone and several hundred in the branch. Of these 21 adults in the cone, 14 (66.7%) were carrying the pitch canker fungus.

C. radiatae or *Pityophthorus* spp. were found infesting 11.4% (n=200) of conelets randomly collected from June, 1990-January, 1991. Overall, 40.7% of attacks were by *Pityophthorus* spp. (n=11) and 59.3% by *C. radiatae* (n=16). The two species did not occur together in any of these conelets. *Pityophthorus* spp. adults attacked more conelets than *C. radiatae* in June (1:0), September (3:1) and October (4:1). However, *C. radiatae* attacks were higher in July (2:0), December (4:0) and January (8:3).

The mean number of beetles per conelet by species and life stage varied from 1.4 *C. radiatae* adults to 2.8 *E. punctulatus* larvae (Table 3). *E. punctulatus* larvae occurred in 37.5% of *C. radiatae*-infested conelets. *E. punctulatus* larvae were not found in these conelets with *Pityophthorus* spp. nor were they found alone. Over one-third of *Pityophthorus* spp. infested conelets contained conspecific larvae. Mean percent contamination per conelet was highest for *E. punctulatus* larvae (53.3%), followed by *Pityophthorus* spp. adults (36.5%), and *C. radiatae* adults (34.4%).

Transmission of *F. subglutinans* to Monterey pine cones under experimental

conditions. When one *C. radiatae* adult was confined to one cone whorl, 38 of the 100 replicates exhibited symptoms of attack. Twelve of the 38 cone whorls attacked (31.6%) contained 2 attacked cones, and 1 exhibited 4 attacks in the same whorl (2 of the 4 cones had progeny). Mean *C. radiatae* attacks per treatment group was 7.6 (SD=1.7, n=5) (Table 4).

Eighty-eight percent of cones attacked by beetles with one or more contaminated species (Groups 1-4) developed pitch canker while only 12.5% of those cones without contaminated beetles (Group 5) developed pitch canker. As the number of attacked cones increased, so did the incidence of pitch canker (Fig. 8). When both beetles were contaminated (Group 4) 10 cones were successfully attacked and 9 of these developed pitch canker. When neither species was contaminated (Group 5), only one of eight attacked cones developed pitch canker. With one exception, where insects were excluded (wounded and unwounded cones) pitch canker did not develop (Groups 8 and 9, Table 4). In the one exception a contaminated *D. auranticella* larva and contaminated *E. punctulatus* larvae were found infesting the cone. When *E. punctulatus* was confined alone on a wounded or unwounded cone, it did not successfully infest the cone or transmit the pitch canker fungus (groups 6 and 7, Table 4). Twenty percent of confined "uncontaminated" *C. radiatae* (C-) and 10% of "uncontaminated *E.*

punctulatus (E-) used as controls tested positive for *F. subglutinans* (Group 5, Table 4).

Hierarchical log-linear analysis showed that there was no significant difference in the number of attacked cones that developed pitch canker among groups of artificially contaminated beetles (Groups 1-4) (Table 4). When neither species was contaminated there was no relationship to the development of pitch canker. The incidence of pitch canker in cones with a confined *C. radiatae* adult was significantly different when compared to cones without *C. radiatae* (96.4% vs. 3.6%, $p=0.00$).

Inter- and intraspecific transmission between *C. radiatae* and *E. punctulatus* under experimental conditions. Interspecific transmission of inoculum in both directions was demonstrated under experimental conditions. The frequency of contaminated *C. radiatae* progeny was greater when a contaminated *E. punctulatus* adult was introduced (74.1%) than when an uncontaminated adult was introduced (25.9%) ($p=0.00$). The frequency of contaminated *E. punctulatus* progeny increased when a contaminated *C. radiatae* adult was compared to an uncontaminated adult (39.1% vs. 60.9%) ($p=0.00$).

The presence of contaminated progeny was dependent upon

introduction of a contaminated parent for both species. Sixty-three percent of *C. radiatae* progeny were contaminated when its parent was contaminated compared to 37.0% when its parent was uncontaminated ($p=0.00$). When a contaminated *E. punctulatus* female was confined, 69.6% of its progeny were contaminated compared to 30.4% when the parent was uncontaminated ($p=0.01$).

C. radiatae and/or *E. punctulatus* progeny were found in 76.4% of the 55 cones attacked by Monterey pine cone beetle. The attacked cones that did not contain *C. radiatae* progeny were abandoned (a gallery was constructed but no eggs were laid). About 42% of the 24 cones without beetle progeny occurred in cone whorls where more than one cone was attacked.

Mean number of progeny per cone was 4.2, 5.9, and 5.2 for *C. radiatae* adults, larvae and pupae, respectively (Table 5). *E. punctulatus* larvae averaged 10.2 per cone. As development from egg to pupae progressed following adult entry into the cone, mean percent contamination with *F. subglutinans* per cone decreased. *C. radiatae* larvae were twice as likely to be contaminated than *E. punctulatus* larvae, but this was not a statistically significant difference.

C. radiatae brood adults had the highest mean percent contamination when both *C. radiatae* and *E. punctulatus* parent adults were artificially

contaminated (72.1%) but it was not a statistically significant difference (Fig. 9). *C. radiatae* larvae and pupae and *E. punctulatus* larvae were most frequently contaminated when adult *E. punctulatus* was artificially contaminated and combined with an uncontaminated *C. radiatae* parent (59.4%, 60.0%, and 36.5%, respectively).

DISCUSSION

There was no significant difference in mean percent contamination with *F. subglutinans* of *C. radiatae* and *E. punctulatus* adults when dissected and reared insects were compared, suggesting that rearing conditions did not significantly affect contamination levels.

Larvae, pupae and teneral adults of *Pityophthorus* spp. were found in cones and conelets. Its presence in cones has not been previously reported in the literature. Adults entered cones from their galleries constructed in the branch tip to the peduncle but often emerged directly from the cone or conelet. This behavior is not unlike that exhibited by several *Conophthorus* spp. (Hedlin et al. 1981; Flores and Bright 1987). Morphological distinctions between these genera are often vague and separation is based primarily on habits. However, several species of *Conophthorus* attack branch tips in addition to cones, i.e. *C. terminalis* Flores and Bright (Flores and Bright

1987), *C. coniperda* Schwarz, *C. resionsae* Hopkins, and *C. banksianae*

McPherson (Hedlin et al. 1981).

C. radiatae and *E. punctulatus* satisfy the criteria for insect vectors of plant pathogens (Leach 1940). *C. radiatae* and *E. punctulatus* consistently carried a high level of inoculum of *F. subglutinans* and contaminated *C. radiatae* and *E. punctulatus* were frequently associated with infected cones under natural conditions. In addition, the strong association between disease symptoms in conelets and the proportion of dissected beetles of both species carrying the fungus strongly suggests a vector relationship with these beetles. When artificially contaminated and confined to their host, *C. radiatae* transmitted the pathogen to healthy cones and progeny acquired the fungus from its parent. Artificially contaminated *E. punctulatus* transmitted this fungus to cones attacked by uncontaminated *C. radiatae*.

More than one female *C. radiatae* may attack the same cone. Cones collected at Mar Monte frequently contained more than one parent female. Cone availability was more limited at Mar Monte than at other sites due to larger populations of *C. radiatae* (over 80% of available cones were infested by *C. radiatae* at this site). In addition, it is not uncommon for the same female to emerge from the first cone she infests to attack a second cone.

Under experimental conditions, a single female confined to a cone whorl attacked 4 cones. Therefore, an individual female can potentially inoculate several cones or conelets. The presence of *C. radiatae* progeny in cones where a single experimentally contaminated parent was confined to a cone whorl suggests that females mate prior to emergence from the brood cone. Adults were removed directly from cones as they emerged for use in this portion of the study and did not have an opportunity to mate outside the cone.

Because *C. radiatae* attacks healthy cones, it may be responsible for initial infections in Monterey pine stands. Fox et al. (1991) suggested that, although engraver beetles transmit the pitch canker fungus to Monterey pines, they may not be an important vector for initial infections. Instead the authors suggest that *Ips* spp. may create a "compounding cycle" leading to increased Monterey pine mortality in some areas by gradually killing diseased trees, and thus increasing their populations. The eventual result may be the infestation of diseased trees followed by attack on nearby healthy trees.

Interspecific transmission of inoculum between *C. radiatae* and *E. punctulatus* was demonstrated under natural and experimental conditions. Contamination of *E. punctulatus* adults trapped in flight peaked in June (Fig. 2) coincident with the beginning of *C. radiatae* pupal development

suggesting opportune timing for interspecific transmission to occur. By August, *C. radiatae* had all reached adulthood and inoculum levels carried by *E. punctulatus* was lowest during this time.

The interspecific transmission of fungal inoculum demonstrated here may have occurred between *Scolytus multistriatus* (Marsham) and *Hylurgopinus rufipes* (Eichhoff), vectors of Dutch elm disease.

Pitch canker remains limited largely to off-site plantings of Monterey pines (Adams 1989). However, spread of the pathogen to native pines may be enhanced by the ability of *C. radiatae* and *E. punctulatus* to transmit inoculum of *F. subglutinans* to each other. *E. punctulatus* also infests Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), knobcone (*Pinus attenuata* Lemm.), and ponderosa pine (*P. ponderosa* Laws) (Furniss and Carolin 1977). These tree species have been shown to be susceptible hosts of pitch canker in greenhouse inoculation studies (Correll et al. 1987). *E. punctulatus* is somewhat analogous to a nest parasite requiring an entrance to cones by another insect. It could potentially inoculate resident insects in susceptible hosts with the pitch canker fungus and thus create new reservoirs for this pathogen.

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Fig. 1. Seasonal life history of *C. radiatae* and *E. punctulatus*.*

*Table is composed of data merged from dissected and reared cones and insects captured on sticky traps. Time line begins with start of attack phase by *C. radiatae* on cones.

Fig. 2. *F. subglutinans* contamination of *C. radiatae*, *Pityophthorus* spp. and *E. punctulatus* captured in sticky traps (sum of 7 different sites) in Santa Cruz and Monterey counties June, 1990-June, 1991.

Fig. 3. Abundance of *C. radiatae* and *E. punctulatus* captured in sticky traps (sum of 7 different sites) in Santa Cruz and Monterey counties June, 1990-June, 1991.

Fig. 4. The proportion of cones containing progeny of *C. radiatae* contaminated with *F. subglutinans* propagules when found in co-habitation with contaminated *E. punctulatus*.*

* Pearson's Chi-square test was used. $p < 0.05$ for all groups. Beetles from reared cones, dissected cones, and both groups combined were analyzed separately.

Fig. 5. Trend in number of 80 infested observational cones that exhibited symptoms of pitch canker, August, 1990-May, 1991.

Fig. 6. Trend in number of 460 observational conelets attacked by *C. radiatae* and *Pityophthorus* spp. June, 1990-May, 1991.

Fig. 7. Proportion of conelets exhibiting pitch canker symptoms with and without contaminated beetles.*

*Pearson's Chi-square test was used for analysis. $p < 0.05$ for all species. Pitch canker was confirmed by isolation of *Fusarium subglutinans* from conelet.

Fig. 8. The number of attacked cones vs. the number of cones that developed pitch canker when artificially contaminated *C. radiatae* and *E. punctulatus* adults were confined on healthy cone whorls.

Fig. 9. Mean *F. subglutinans* contamination of progeny per cone from artificially contaminated adults of *C. radiatae* and *E. punctulatus* confined to healthy cones by treatment group.*

* "C" = *C. radiatae*, "E" = *E. punctulatus*, "+" = contaminated insect, "-" = uncontaminated insect.

Table 1. Experimental design of inoculation studies: beetles artificially contaminated with *F. subglutinans* and confined on healthy cone whorls to test the capacity of these insects to vector *F. subglutinans*.*

<u>Group</u>	<u>Insects</u>
1	C+ alone
2	C- X E+
3	C+ X E+
4	C+ X E-
5	C- X E-
6	E+ alone on wounded cones
7	E+ alone unwounded cones
8	Wounded cones, exclusion cages
9	Unwounded cones, exclusion cages

*C = *C. radiatae*, E = *E. punctulatus*, "+" = experimentally contaminated insect, "-" = uncontaminated insect.

Table 2. The mean number and percent contamination per conelet of resident beetles dissected from infested observational conelets, August, 1990-May, 1991.

<u>Species</u>	<u>Mean No. of Beetles</u>	<u>Mean Percent Contamination</u>
<i>C. radiatae</i> adults	1 (9) +/- 0	33.3% +/- 0.5
<i>Pityophthorus</i> spp. adults	3.0 (31) +/- 2.3	40.7% +/- 0.5
<i>Pityophthorus</i> spp. larvae	3.6 (7) +/- 4.7	9.7% +/- 0.1
<i>E. punctulatus</i> larvae	4.0 (17) +/- 3.1	13.2% +/- 0.3

Table 3. *F. subglutinans* contamination of conelets attacked by *C. radiatae*, *Pityophthorus* spp., and *E. punctulatus* recovered from 200 randomly collected conelets, June, 1990-January, 1991.

<u>Species</u>	<u>Mean No. of Beetles</u>	<u>Mean Percent Contamination</u>
<i>C. radiatae</i> adults	1.4 (16) +/- 0.9	34.4% +/- 0.4
<i>Pityophthorus</i> spp. adults	2.2 (9) +/- 1.9	36.5% +/- 0.5
<i>Pityophthorus</i> spp. larvae	2.0 (4) +/- 1.4	12.5% +/- 0.3
<i>E. punctulatus</i> Larvae	2.8 (6) +/- 2.6	53.3% +/- 0.5

Table 4. Frequency of transmission of *F. subglutinans* by *C. radiatae* and *E. punctulatus* artificially contaminated by this fungus in various species combinations.

<u>Treatment Group*</u>	<u>No. of Cones With Pitch Canker/ No. of Attacks</u>
1. C+	5/6
2. C+ E-	5/6
3. C- E+	7/8
4. C+ E+	9/10
5. C- E-	1/8
6. E+ Wounded	0/0
7. E+ Unwounded	0/0
8. Wounded	0/0
9. Unwounded	1/1

*See Table 1 for species designation.

Table 5. Progency produced by adults artificially contaminated with *F. suglutinans*.

<u>Species</u>	<u>Mean No. of Progeny</u>	<u>Mean Percent Contamination</u>
<i>C. radiatae</i> adults	4.2 (26) +/- 8.6	62.2% +/- 0.5
<i>C. radiatae</i> larvae	5.9 (15) +/- 6.0	40.7% +/- 0.4
<i>C. radiatae</i> pupae	5.2 (14) +/- 5.1	24.1% +/- 0.4
<i>E. punctulatus</i> larvae	10.2 (42) +/- 8.3	20.6% +/- 0.2