

Secondary insects and diseases contribute to sudden aspen decline in southwestern Colorado, USA

Suzanne Bethers Marchetti, James J. Worrall, and Thomas Eager

Abstract: Reports of drought-associated forest mortality have increased around the world, but the mechanisms of mortality are rarely direct in nature. Biotic agents may kill trees that could otherwise recover and can perpetuate and expand mortality after the stress is relieved. Sudden aspen decline (SAD) has caused rapid, widespread branch dieback and mortality of quaking aspen (*Populus tremuloides* Michx.). We compared insects and diseases in 162 damaged and neighboring healthy plots to determine contributing factors and their ecological roles. Cytospora canker, bronze poplar borer, and aspen bark beetles were the most common agents in damaged plots and correlated with crown loss and other factors related to SAD. This was the first documented outbreak of *Trypophloeus populi*, an aspen bark beetle. As bark beetles and bronze poplar borer increased in damaged stands, they tended to attack trees with healthier crowns. Environmental stress may have directly affected the success of these agents by increasing host susceptibility followed by a density-dependent increase in the insects' invasive ability. In contrast, Cytospora canker had an identical relationship to crown loss in healthy versus damaged plots, suggesting that it was not limited by inoculum but responded to host susceptibility. Most other pathogens and insects contributed little to SAD and appear to be primary or weakening agents. The biotic agents of mortality in a decline differ greatly from primary agents and play complex and varied roles in healthy versus declining stands.

Résumé : Les rapports de mortalité des forêts associée à la sécheresse ont augmenté partout dans le monde mais les mécanismes responsables de la mortalité sont rarement de nature directe. Des agents biotiques peuvent tuer des arbres qui pourraient par ailleurs récupérer et peuvent perpétuer et augmenter la mortalité après que le stress soit disparu. Le dépérissement soudain du tremble (DST) est responsable de la mort en cime et de la mortalité rapide et généralisée du peuplier faux-tremble (*Populus tremuloides* Michx.). Nous avons comparé les maladies et les insectes présents dans 162 placettes touchées par le dépérissement appariées à des placettes saines avoisinantes pour déterminer les facteurs contributifs et leurs rôles écologiques. Le chancre cytosporéen, l'agrile du peuplier et les scolytes du tremble étaient les agents les plus fréquents dans les placettes touchées par le dépérissement et ils étaient corrélés avec la mort en cime et d'autres facteurs reliés au DST. Il s'agissait de la première épidémie documentée de *Trypophloeus populi*, un scolyte du tremble. À mesure que les populations de scolytes du tremble et d'agrile du peuplier augmentaient dans les peuplements touchés par le dépérissement, ces insectes avaient tendance à s'attaquer à des arbres dont la cime était plus saine. Un stress environnemental pourrait avoir directement influencé le succès de ces agents en provoquant une augmentation de la sensibilité de l'hôte, suivie d'une augmentation de la capacité invasive des insectes qui serait dépendante de la densité. Au contraire, le chancre cytosporéen avait la même relation avec la mort en cime que les placettes soient touchées ou non par le dépérissement, ce qui indique que son incidence ne dépend pas de la quantité d'inoculum mais plutôt de la sensibilité de l'hôte. La plupart des autres pathogènes et insectes contribuaient peu au DST et semblent être des agents primaires ou qui affaiblissent leur hôte. Les causes biotiques de mortalité dans le cas du dépérissement diffèrent grandement des agents primaires et jouent des rôles complexes et variés dans les peuplements sains comparativement aux peuplements qui dépérissent.

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Introduction

Increasing temperatures worldwide and more frequent and severe droughts projected in many regions in the coming decades (Core Writing Team 2008) will increase moisture stress in forests, especially during extreme years. Indeed, reports of apparently heat- and drought-incited episodes of tree mortality have increased markedly around the world, even in envi-

ronments that are not normally considered water limited (Allen et al. 2010).

Progress is being made in understanding physiological mechanisms of drought stress leading to mortality (McDowell 2011). However, it is rare in nature that trees die directly from abiotic disease or injury such as drought. Instead, insects and pathogens are typically the proximate agents of mortality (Houston 1992; Desprez-Loustau et al. 2006).

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Although it is sometimes assumed that these pathogens and insects simply accompany mortality that would occur without them, their role is often more important. For example, these agents may kill trees that otherwise would recover when cooler temperatures or precipitation return (Houston 1992). Also, the agents build in population and inoculum potential increases on stressed trees, which increases aggressive behavior so that they are able to kill healthier trees (Furniss and Carolin 1977), expanding the mortality event in time and space.

Although the biotic agents that contribute to declines respond positively to stressed hosts, that is not true of all pathogens and insects. We recognize three main types of damage agents in trees. (i) Primary agents are pathogens and insects that can attack and kill vigorous trees. Some, mostly obligate parasites, actually fare better on vigorous than on stressed hosts (Schoeneweiss 1975). (ii) Secondary agents attack and kill already stressed trees but normally cannot successfully attack vigorous trees. As noted above, some are able to transition from secondary to primary behavior when sufficient resources are available. (iii) Weakening agents diminish growth and may cause vigorous trees to deteriorate but do not commonly cause direct mortality.

A multiyear, severe, and very warm drought in the southwestern United States peaked in 2002 (Pielke et al. 2005; Rehfeldt et al. 2009). It quickly led to a severe outbreak of the bark beetle *Ips confusus* that caused widespread mortality of pinyon pine (*Pinus edulis* Engelm.) (Breshears et al. 2005) as well as a marked increase in mortality in mixed conifer and ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C. Lawson) forests involving various bark beetles (Ganey and Vojta 2011). It also incited sudden aspen decline (SAD) of quaking aspen (*Populus tremuloides* Michx.) in Colorado and neighboring states (Rehfeldt et al. 2009; Worrall et al. 2008, 2010). SAD was first observed in 2004, several years after the mortality in *P. edulis*, and the biotic agents involved were more varied.

SAD has become a significant issue in the Southern Rocky Mountains and the southwestern United States (Bartos 2008; Fairweather et al. 2008; Worrall et al. 2008; Rehfeldt et al. 2009). By 2008, it occurred on approximately 19% of the quaking aspen cover type in the national forests of southwestern Colorado (Worrall et al. 2010). SAD is characterized in part by rapid, synchronous branch dieback, crown thinning, and mortality of aspen stems on a landscape scale.

SAD fits well into a decline-disease concept with predisposing, inciting, and contributing factors (Manion and LaChance 1992; Worrall et al. 2008). Predisposing factors are slowly changing conditions, such as age, site and stand conditions, and long-term climate. Predisposing factors linked to SAD include open stands, low elevations, exposed slope positions, and southerly aspects (Worrall et al. 2008, 2010). Inciting factors are short-term physiological or biological factors that cause acute stress, including drought, insect defoliation, frost, and air pollution. With these factors alone, trees may recover quickly, but recovery is much slower if the population is affected by predisposing factors. The inciting factor for SAD was the 2002 drought. Finally, contributing factors kill trees that have been debilitated by predisposing and inciting factors. Contributing factors are most often secondary pathogens and insects.

Initial observations in the southwestern United States suggested that secondary agents were frequent in declining aspen stands and could be the contributing factors in SAD, but few quantitative data were presented (Fairweather et al. 2008; Worrall et al. 2008). In addition, repeated defoliator outbreaks have been noted as inciting and (or) contributing factors in other recent aspen declines in Canada (Hogg et al. 2002; Brandt et al. 2003) and in Arizona (Fairweather et al. 2008).

Our objectives were to (i) determine what agents were important contributing factors in SAD in southwestern Colorado, (ii) detail some of the ecology and community structure of these and associated agents, which in many cases are not well documented, (iii) describe the patterns and processes of aspen mortality with respect to the agents, and (iv) compare the behavior of these agents in healthy and damaged stands.

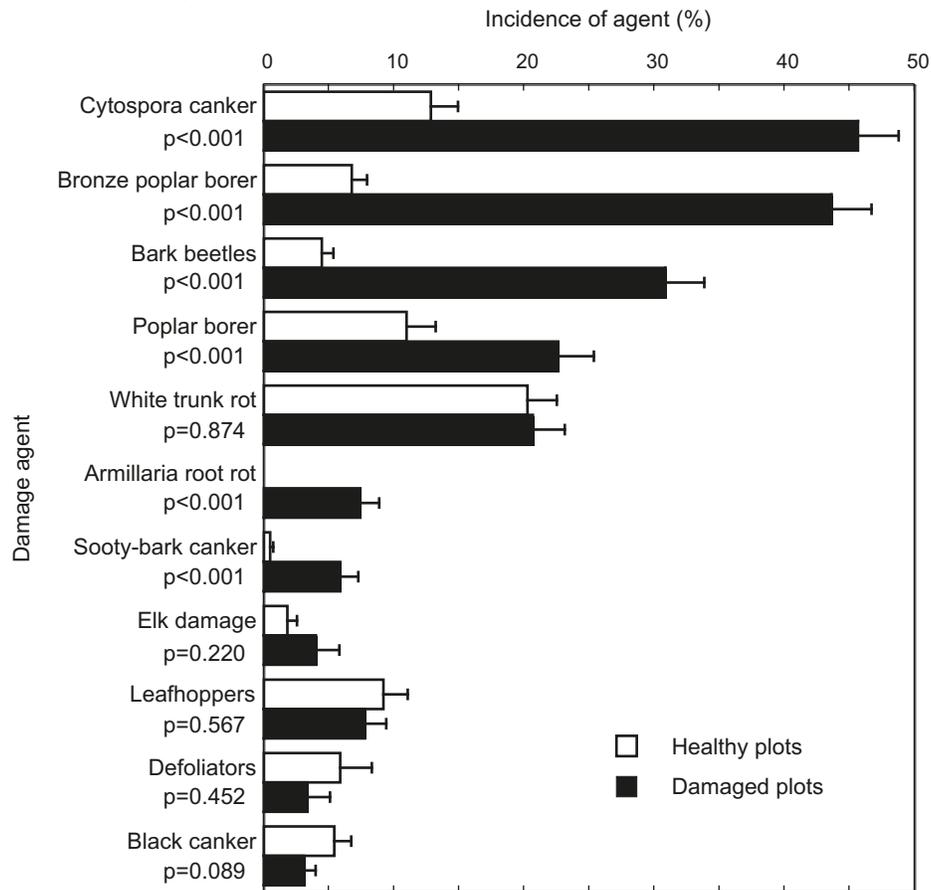
Methods

This study was part of an extensive survey of SAD in southwestern Colorado in 2007 and 2008. Sampling methods are detailed in Worrall et al. (2010). In brief, we sampled 81 paired plots of damaged and healthy quaking aspen on four national forests of southwestern Colorado including the Grand Mesa, Uncompahgre, and the Gunnison National Forests as well as the Mancos-Dolores District of the San Juan National Forest. Aerial surveys from 2006 and 2007 were used to randomly select points in polygons with SAD. A point in an adjacent healthy polygon, >200 m from the damaged plot, was selected for comparison. This paired plot selection was meant to eliminate confounding factors, like those frequently observed with changes in elevation, and focus on those factors unique between healthy and damaged plots.

Damaged plots had >25% mean recent crown loss (RCL), while healthy plots had ≤25%. Twenty-five percent was the approximate minimum threshold for detecting SAD damage by aerial survey (Worrall et al. 2010). RCL was estimated as the percentage of foliage that was lost, either due to branch and twig dieback or due to crown thinning. Only recent loss was included in the estimate; dead branches without fine twigs or with <50% intact bark were not considered part of the crown.

At each point, we recorded the location and shrub cover. Trees ≥12 cm diameter at breast height (DBH) were selected using variable-radius prism plots; these were measured and RCL was recorded for each live or recently dead aspen. Four dominant or codominant aspen stems were selected for increment coring and height measurements, avoiding those with stem decay. Each live and recently dead aspen was examined for insects, diseases, and injury. Old snags (those trees without fine twigs or <50% intact bark) were not surveyed for damage agents because features by which agents are identified were obscured in older mortality. Elk damage was only recorded when feeding injury penetrated to the wood, and trees were inspected for Armillaria root disease (caused by *Armillaria* spp.) only when they had high crown loss, were recently dead, or when basal decay was evident. Aspen bark beetles (*Proccryphalus mucronatus* and *Trypophloeus populi*)

Fig. 1. Mean incidence (% of trees affected), with standard error, of damage agents in healthy and damaged aspen plots. *p* values derived from paired *t* tests; *n* = 81 each of damaged and healthy plots.



and other insects were collected for identification when they were found on plots.

Coarse aspen roots (≥ 4 mm diameter) were measured with a root trench 3 m long and 20 cm deep (Shepperd et al. 2001). The diameter and condition (live or dead) of each intercepted aspen root were recorded. Tree slenderness was calculated as the ratio of height to DBH (Wang et al. 1998). Elevation was calculated using digital elevation data with a resolution of 3 arc-seconds. As an index of moisture availability, we used a climate moisture index (CMI) based on the simplified Penman–Monteith approach in Hogg (1997) as described by Worrall et al. (2010). We calculated CMI for the 2002 hydrological year (October 2001 – September 2002), the driest and hottest year of the drought (Pielke et al. 2005). Estimates of areas of forest defoliated by western tent caterpillar (*Malacosoma californicum*), large aspen tortrix (*Choristoneura conflictana*), and generic defoliators from 1994 to 2004 recorded during aerial surveys conducted in the Rocky Mountain Region of the US Forest Service (www.fs.usda.gov/goto/r2/fh, ADS) were totaled annually within aspen cover type on the national forests included in this study.

Statistical analysis

RCL was used as the primary response variable representing degree of damage to trees and plots; it was examined and compared with other variables in two ways. First, plot RCL

was calculated as an average of the live and recently dead aspen trees in each plot. Second, all trees were grouped into RCL classes in 5% increments without regard to plot; this was termed tree RCL. Trees were also grouped by DBH in 5 cm increments. With each agent noted as present or absent on live and recently dead aspen trees, we calculated incidence as the percentage of trees identified with the agent or agent damage, such as a gallery.

Statistical analyses were conducted using Statistix 9.0 (Anonymous 2008). Pearson correlation, linear regression, quadratic regression, and paired *t* tests were used. We also compared the slope and elevation of regressions in healthy versus damaged plots. Classification was conducted to find associations among agents using TWINSpan version 2.3 (Hill and Šmilauer 2005). In addition to classifying plots by presence and absence of agents, the incidence of agents in a plot was incorporated using the “pseudo species” tool to represent agent frequency levels at 20%, 40%, 60%, or 80% of the aspen stems affected in the plot. Groups were recombined if there were less than three plots in a group. One group contained most of the damaged plots; this was divided further into four subgroups.

Results

We examined 2240 live and recently dead quaking aspen stems and found 3088 occurrences of agent damage. The

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Table 1. Pearson correlation coefficients among damage agents based on incidence in aspen plots.

	Cytospora canker	Bronze poplar borer	Bark beetles	Armillaria root rot	Sooty-bark canker	Poplar borer	White trunk rot
Bronze poplar borer	0.57***						
Bark beetles	0.59***	0.64***					
Armillaria root rot	0.38***	0.40***	0.34***				
Sooty-bark canker	0.03	0.37***	0.26***	0.09			
Poplar borer	0.20*	0.09	0.16*	0.04	-0.04		
White trunk rot	-0.11	-0.07	-0.12	0.07	0.06	-0.16*	
Leafhoppers	-0.09	-0.08	0.01	-0.17*	-0.06	-0.02	-0.02

Note: Significance: * $p \leq 0.05$, *** $p < 0.001$; $n = 162$ plots.

Table 2. Significant Pearson correlation coefficients of damage agents with aspen site and stand factors.

Plot characteristic	Bronze poplar	Bark beetles	Cytospora canker	Armillaria root rot	Poplar borer	Sooty-bark canker	Leafhoppers
Root mortality	0.42***	0.35***	0.38***	0.16*			-0.23**
Recent crown loss	0.79***	0.64***	0.68***	0.45***	0.20*	0.27***	-0.16*
Tree slenderness	-0.30***	-0.35***	-0.34***	-0.23**	-0.19*		
Moisture index, 2002			-0.17*	-0.16*		0.23**	0.40***
Basal area		-0.21*	-0.17*		-0.29***		
Shrub cover					0.25**	-0.19*	-0.16*

Note: Significance: * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$; $n = 162$ plots.

most common agents were Cytospora canker (usually caused by *Valsa sordida*), bronze poplar borer (*Agrilus liragus*), poplar borer (*Saperda calcarata*), aspen bark beetles (*Procyphallus mucronatus* and *Trypophloeus populi*), white trunk rot (caused by *Phellinus tremulae*), Armillaria root rot (caused by *Armillaria* spp.), sooty-bark canker (caused by *Encoelia pruinosa*), defoliators (various), elk herbivory, leafhoppers (*Idiocerus* spp.), and black canker (caused by *Ceratocystis populicola*) (Fig. 1). Many other damage agents were recorded but with <1% mean incidence in plots; these included galls, white mottled root rot (caused by *Ganoderma applanatum*), Cryptosphaeria canker (caused by *Cryptosphaeria lignyota*), Hypoxylon canker (caused by *Entoleuca mammatum*), sunscald, and other abiotic damage.

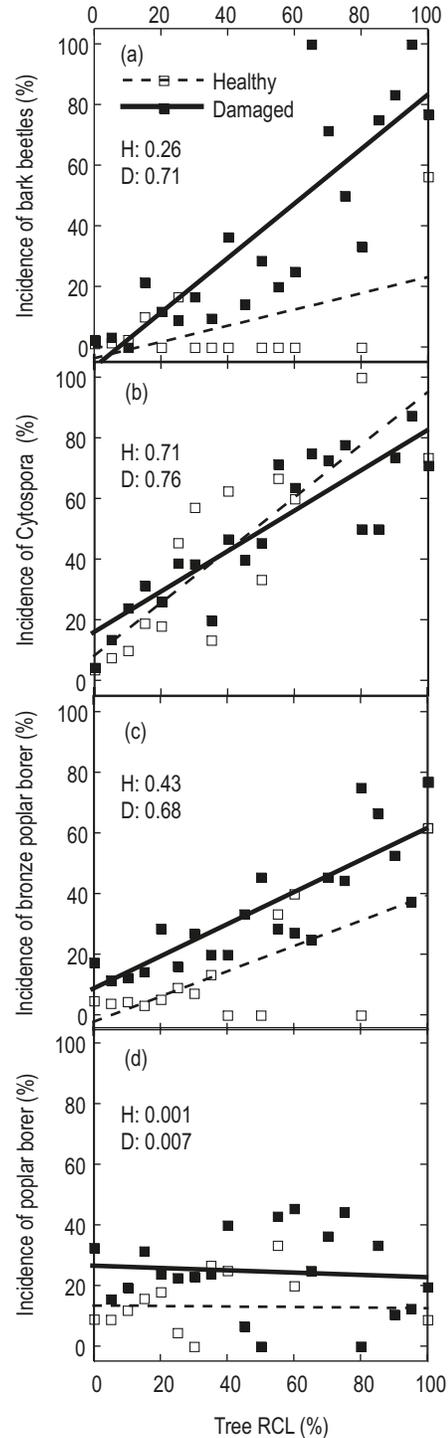
Secondary agents, including Cytospora canker, bronze poplar borer, aspen bark beetles, and poplar borer, had the highest incidence in damaged plots and were significantly more frequent there than in healthy plots (Fig. 1). White trunk rot was also common, but the incidence in damaged and healthy plots was nearly the same. Armillaria root rot and sooty-bark canker were significantly more frequent in damaged plots compared with healthy plots but much less common than the above agents. The remaining agents were infrequent and had no significant differences between damaged and healthy plots.

Defoliators (aspen tortrix, leaf rollers, and western tent caterpillar) had an average incidence of <6% in plots; healthy and damaged plots were not significantly different. Defoliation in plots was mild to moderate. A compilation of 10 years of the aerial survey in the study area prior to 2004, when SAD was first observed in southwestern Colorado, showed no defoliation by aspen tortrix or western tent caterpillar, which supported our observations during that time. Aerial surveys included few notations of generic defoliation (unknown agent); defoliation was recorded in relatively minute areas in 2000 (7 ha), 2002 (56 ha), and 2003 (66 ha). During the decline, from 2004 to 2008, there were also small, isolated occurrences of defoliators including 842 ha of aspen tortrix in 2005 and 170 ha of tent caterpillar in 2006. There were 68 047 ha of SAD in 2008 (Worrall et al. 2010); the total defoliated area noted by aerial survey is <2% of that value.

Cytospora canker, bronze poplar borer, and bark beetles tended to occur together in plots, as demonstrated by strong correlations among them (Table 1). Armillaria root rot was also significantly correlated with these three agents but with lower correlation coefficients. Sooty-bark canker was correlated with bronze poplar borer and bark beetles but not with Cytospora canker. Poplar borer was only weakly correlated with bark beetles and Cytospora canker.

Cytospora canker, bronze poplar borer, and bark beetles were positively correlated with root mortality and plot RCL and negatively correlated with tree slenderness (Table 2). Poplar borer and sooty-bark canker were not significantly correlated with root mortality but were weakly associated with plot RCL. Armillaria followed significant trends similar to Cytospora canker, bronze poplar borer, and the aspen bark beetles, but the correlation coefficients were lower. The incidence of sooty-bark canker and leafhoppers increased with 2002 CMI values of plots (high values indicate more available moisture) and decreased with shrub cover. In contrast,

Fig. 2. Incidence of the four most common damage agents versus tree RCL in healthy and damaged aspen plots. Crown loss was estimated in 5% increments in the field, and trees were grouped by these 5% increments; $n = 2240$ live and recently dead trees. H and D represent R^2 from regressions of healthy and damaged plots, respectively.



Cytospora canker and Armillaria were negatively correlated with CMI and poplar borer increased with shrub cover.

The agents with the highest incidences in damaged plots differed substantially in their relationships to tree RCL (Fig. 2). The increase of bark beetles with tree RCL was sig-

Table 3. Mean incidence (% of trees affected) of damage agents in aspen plots classified by agent communities using TWINSpan.

	Group										Total agent incidence (%)
	1	2	3	4	5	6	6a	6b	6c	6d	
Sooty-bark canker	0.3	0.0	0.0	0.0	2.9	4.9	2.8	2.3	6.1	6.7	3.2
Armilaria root rot	0.0	0.8	0.0	0.0	5.0	6.1	2.0	0.4	7.3	10.8	4.1
Bronze poplar borer	2.6	5.3	7.5	6.0	13.8	38.7	10.8	31.3	40.3	59.6	25.2
Cytospora canker	2.7	9.2	9.3	5.5	6.6	46.2	27.6	30.4	47.5	65.8	29.2
Bark beetles	1.9	3.0	4.6	0.8	4.3	28.6	16.1	13.6	19.5	51.2	17.6
Poplar borer	2.2	2.7	47.4	53.4	4.3	21.3	15.3	42.5	4.8	25.7	16.8
White trunk rot	4.4	19.9	14.3	10.9	56.4	17.7	19.9	14.7	30.6	8.7	20.5
Leafhoppers	4.4	22.4	3.1	3.7	4.6	7.3	6.6	19.5	2.7	4.5	8.5
Elk damage	0.6	4.9	1.5	0.0	0.7	3.6	0.0	5.8	4.4	4.2	2.9
Defoliators	0.0	1.4	97.1	1.4	0.3	3.3	0.0	12.5	3.3	0.6	4.6
Black canker	4.4	3.8	0.0	14.9	5.3	3.5	3.4	2.9	4.6	3.0	4.2
Plot count	18	22	4	7	18	93	21	17	23	32	162
Damaged plots (%)	6	0	0	29	33	76	48	71	78	97	50

Note: The bottom row indicates the percentage of plots in each group that were damaged (>25% RCL).

nificantly steeper in damaged plots than in healthy plots ($p = 0.01$) (Fig. 2a). In healthy plots, there was no consistent increase and most aspen bark beetle galleries were found in dead trees (100% RCL). The pattern of increase of Cytospora canker with tree RCL was indistinguishable between healthy and damaged plots, with incidence increasing linearly (at a slope of 0.9) in both plot types (Fig. 2b). The incidence of bronze poplar borer with tree RCL followed a similar slope in healthy and damaged plots ($p = 0.468$) (Fig. 2c), but the incidence was significantly lower in trees from healthy plots compared with damaged plots ($p = 0.002$). The incidence of poplar borer versus tree RCL (Fig. 2d) contrasted with that of the other three major damage agents. Poplar borer was more frequent in damaged than in healthy plots ($p < 0.001$), but there was no relationship between tree RCL and the incidence of poplar borer.

An initial classification of plots into agent communities resulted in six agent communities among 162 plots (Table 3). Group 6 contained 93 plots; these were mostly damaged and with high levels of secondary agents; this group was reclassified into four subgroups (6a–6d). The resulting nine agent communities differed significantly in plot RCL; those plots with the highest levels of secondary agents had the highest average plot RCL (Fig. 3).

Groups 1–3 were mainly comprised of healthy plots, but group 1 had the lowest levels of damage agents with <5% incidence of any agent. Group 2 was dominated by leafhoppers (22%) and some white trunk rot (20%), and group 3 had a high incidence of insect defoliation (97%), but neither group included any damaged plots. Groups 4 and 5 were a mix of healthy and damaged plots and were characterized by poplar borer (53%) and white trunk rot (56%), respectively. Of the 93 plots in group 6, 22 were “healthy” plots, meaning that they had $\leq 25\%$ RCL. The four subgroups of group 6 were differentiated by the incidence of secondary agents (Table 3). Group 6a had a mix of healthy and damaged plots and had the lowest plot RCL and agent levels among the subgroups. The greatest proportion of damaged plots was in group 6d, which was characterized by high incidence of Cytospora canker, bronze poplar borer, and bark beetles.

The study plots ranged in elevation from 2300 to 3300 m. The incidence of white trunk rot was negatively related to elevation (Fig. 4). Sooty-bark canker peaked at midelevations around 2800 m. The remaining agents showed no significant trend in incidence with elevation.

Incidence of some agents varied with tree diameter (Fig. 5), although generally, these agents could be found on trees in the smallest and largest size classes. In healthy plots, the incidence of aspen bark beetles and black canker increased slightly but significantly with tree size. White trunk rot increased substantially in trees >40 cm DBH. In contrast, the incidence of poplar borer in healthy plots was highest in the smallest trees and decreased to nearly 5% in trees >25 cm DBH. But in damaged plots, the incidence of bronze poplar borer increased linearly with DBH, as did that of aspen bark beetles and white trunk rot. Sooty-bark canker increased significantly with DBH class, and the incidence of sooty-bark canker also increased with mean DBH of damaged plots ($R^2 = 0.087$, $p = 0.008$). The incidence of elk damage within damaged plots was negatively related to plot DBH ($R^2 = 0.107$, $p = 0.003$), with most damage occurring on trees

Fig. 3. Mean plot RCL of the plot groups classified by agent community in TWINSPAN. Error bars indicate standard error; letters above error bars indicate significant differences from Tukey's (HSD) comparison of means. See Table 3 for the number of plots in each group.

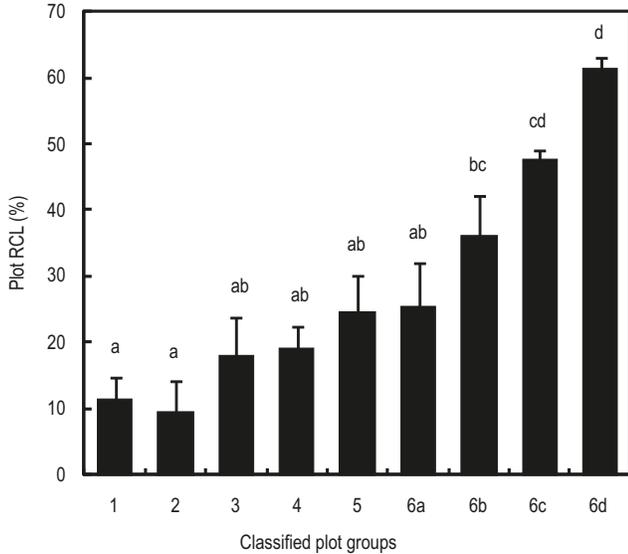
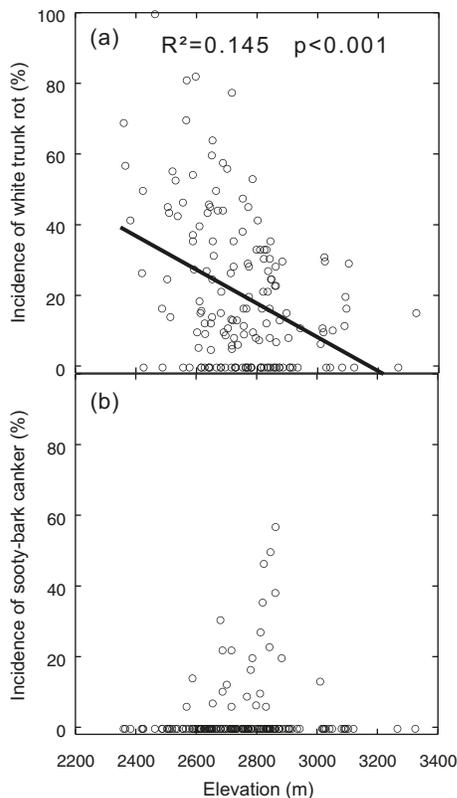


Fig. 4. (a) Significant linear regression of white trunk rot incidence versus elevation in aspen plots; $n = 162$ plots. (b) Sooty-bark canker incidence peaked at middle elevations.



<25 cm DBH. The other recorded agents showed no significant trends with DBH. Within plots, bark beetles, poplar borer, white trunk rot, and black canker occurred on trees

that were significantly larger than the other trees in the plot (data not shown).

Of 184 adult bark beetles collected and identified from the study areas, 78% were *T. populi* and the remainder were *P. mucronatus*. Ninety-three bark beetles were collected from the plots. Of 79 beetles from damaged plots, 79% were *T. populi*. Beetles were only collected from one healthy plot; all 14 were *P. mucronatus*. Collections from late May to early July were mostly *P. mucronatus*; *T. populi* dominated collections from mid-July to the end of August. Incidental to this study, from 2005 to 2008, we observed multiple cases of mass attacks of bark beetles on large aspen stems that appeared healthy, with little RCL. Gallery patterns were typical of *T. populi*.

Discussion

Cytospora canker, bronze poplar borer, and aspen bark beetles appear to be the most important agents associated with SAD. They were the dominant agents in damaged plots, were much less common in healthy plots, and were strongly correlated with one another. They were also correlated with the same stand factors associated with SAD: root mortality, RCL, and (negatively) tree slenderness. Among the agents previously noted in SAD stands (Fairweather et al. 2008; Worrall et al. 2008), this study focuses attention on these as the most important agents contributing to mortality in southwestern Colorado.

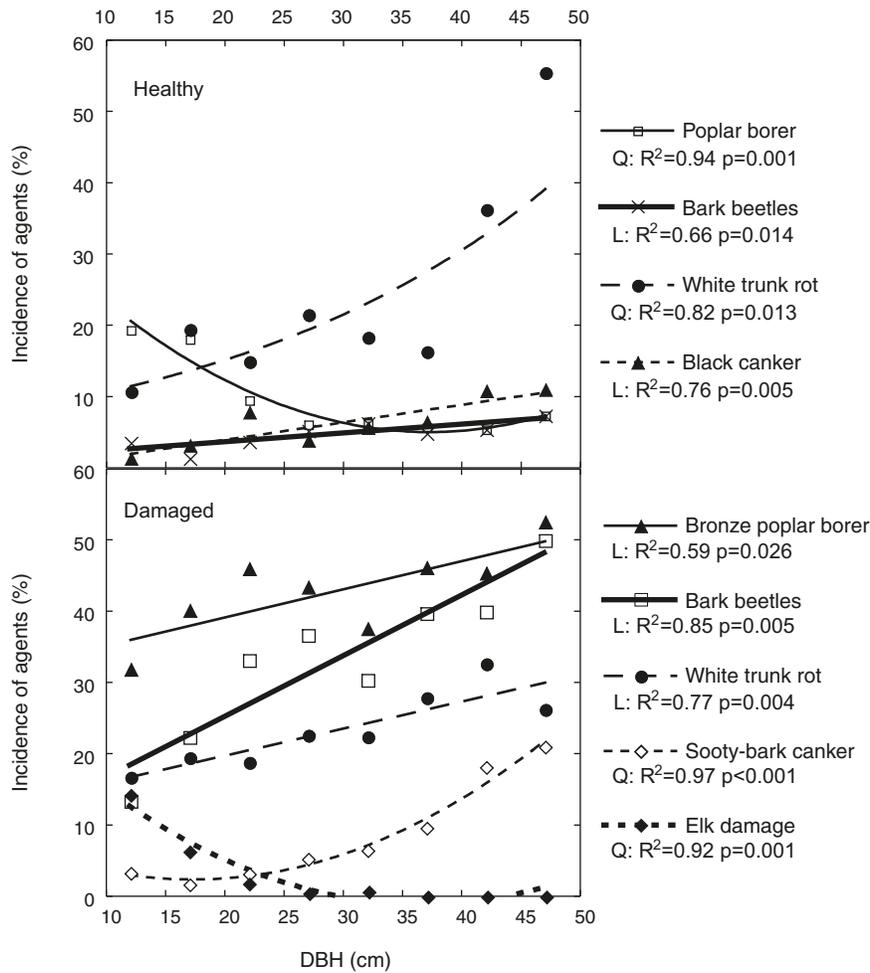
Cytospora canker and tree RCL were tightly correlated in both healthy and damaged plots. Because the disease is present in most trees with crown symptoms, even in healthy stands, inoculum is apparently available for all susceptible trees and is not a limiting factor in infection and disease expression. Chapela (1989) found latent infections of *Cytospora chrysosperma* in healthy aspen bark sufficient to cause disease when drought occurred. The importance of Cytospora canker in this drought-incited decline is consistent with what is already known about Cytospora canker, which rapidly invades trees exposed to moisture stress (McIntyre et al. 1996). The steep slope of the regression line between tree RCL and the incidence of Cytospora canker is consistent with the effects of the disease in killing phloem and cambium, thereby killing all or part of the tree. It was common in all size classes of trees and across elevations. It appears to be a ubiquitous and important agent in SAD and in aspen mortality overall.

Like Cytospora canker, bark beetles appeared to play an important role in the mortality of aspen in damaged plots. However, bark beetles attacked few trees from healthy plots unless they were dead. This could suggest that unlike inoculum of Cytospora canker, the local population size of the beetles can be an important limiting factor for successful infestation of a tree. Perhaps these bark beetles only aggregate in areas with a critical mass of declining trees. There was a preference in bark beetles for large-diameter trees in both healthy and damaged stands, which is consistent with other species of bark beetles (Hopping and Beall 1948).

The two species of aspen bark beetles play different roles in SAD. The majority of the bark beetles collected from damaged plots were *T. populi*. In healthy plots, only *P. mucronatus* was collected, and bark beetle galleries were fre-

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Fig. 5. Incidence of damage agents versus tree DBH class mean in healthy and damaged aspen plots. Quadratic (Q) or linear (L) regressions noted, with R^2 and p values listed; $n = 2240$ live and recently dead trees. Agents not shown had no significant relationship with DBH ($p > 0.05$).



quent only on dead trees. Although they are both considered secondary agents, these beetles have very different behavior in host trees. In a study by Petty (1977), *T. populi* was more aggressive and attacked trees that were stressed but still green, while *P. mucronatus* was found mostly in dead bark on trees that were nearly or completely dead. Our results are consistent with Petty's (1977), but *T. populi* has behaved even more aggressively in association with SAD than in Petty's (1977) study. We noted that, during this study, *T. populi* mass-attacked large, apparently healthy aspen. This behavior has not been reported previously and is one of the novel features of SAD. The ready availability of potential hosts (drought-stressed aspens) undoubtedly contributed to the general increase in *T. populi* populations. Our plot data constitute the first documentation of an outbreak of these aspen bark beetles.

The incidence of bronze poplar borer increased with tree RCL, but somewhat more so and more consistently in damaged plots than in healthy plots. This suggests that successful infestation by bronze poplar borer is also determined by population size, making them more successful in damaged plots because they are at higher densities. Barter (1965) found that bronze poplar borer could successfully infest healthy trees only when there were high borer populations to overwhelm a

healthy tree's defenses. The borer's success is often dependent on the initial weakness of the tree; a vigorous tree will confine the bronze poplar borer to a tight zigzag, while a declining tree will allow for long, straighter galleries affecting more cambial area (Solomon 1995). Bronze poplar borer was common across elevations and was present more frequently in large trees, especially in damaged stands. As the larvae feed in the phloem, cambium, and outer xylem, they disrupt nutrient transport, causing branch dieback (Barter 1965). Because the larvae feed primarily in the cambium region, larger-diameter trees can sustain greater broods of larvae before killing the tree, perhaps making large trees a favorable target.

It is difficult to clarify the relationship between SAD and poplar borer. Although it was frequently observed in damaged plots, it was not well correlated with the other main SAD agents, tree RCL, or root mortality. Its association with SAD could be due to an overlap in site characteristics of SAD and favorable sites for poplar borer. Poplar borer was associated with several factors indicating open stands: high shrub cover, low basal area, and lower tree slenderness, which is consistent with the literature on poplar borer (Graham and Mason 1958; Brandt et al. 2003); SAD is also common in open stands. There may be other reasons why poplar

borer is not associated with crown loss. Because the life cycle of poplar borer is 2–3 years (Furniss and Carolin 1977), it may be present in trees for long periods, causing delayed crown symptoms. Also, poplar borer larvae feed primarily in the xylem rather than in the vascular cambium. Because xylem can tolerate biotic attack and remain functional for multiple years, poplar borer may not directly or quickly cause substantial crown symptoms. Even at high levels of infestation, a tree is rarely killed but is more susceptible to wind breakage (Solomon 1995). In healthy plots, poplar borer acted as a thinning agent, attacking small-diameter trees more frequently than large-diameter trees.

Sooty-bark canker, an aggressive disease and a dominant killer of vigorous aspen in Colorado (Juzwik et al. 1978), had significantly greater incidence in damaged than in healthy plots. Trees with sooty-bark canker were weakened and therefore suitable for secondary agents such as bronze poplar borer and bark beetles. However, there are several reasons to believe that sooty-bark canker does not play a substantial role in SAD: (i) it had low mean incidence in damaged plots (5.8%), (ii) it was not associated with dry, open stands, as is SAD; rather, it occurred in more mesic sites (higher CMI) with low shrub cover, (iii) it was not correlated with root mortality, which is part of the SAD syndrome, and (iv) unlike SAD, it was associated with plots of high mean DBH. Finally, it is the most important killer of aspen in normal times (Hinds 1964; Juzwik et al. 1978), so it should be expected that it would appear in some dead and dying trees in this study. Thus, sooty-bark canker is not a contributing factor in SAD, but it is still an important mortality agent in aspen.

Armillaria root disease has been considered a primary pathogen on aspen (Brandt et al. 2003) and as a secondary agent on stressed aspen (Frey et al. 2004). Armillaria was found at low incidence but followed trends similar to bronze poplar borer, Cytospora canker, and bark beetles. It was positively correlated with those agents and with SAD site factors. However, our methodology for detecting Armillaria was limited to checking roots and butts on trees with high RCL and (or) obvious basal decay to avoid damage to healthy trees. Therefore, we can make no conclusions about its presence in trees with healthy crowns. More investigation is needed to determine whether Armillaria plays an important role in SAD.

Impact of elk on mature trees was low, although there were isolated areas of heavy damage. Elk barking was often conspicuous but was not recorded unless it reached the wood. We did not record damage on trees <12 cm in diameter, which we considered regeneration. Regeneration of aspen stands is often limited by ungulate herbivory, especially by elk or cattle (Fitzgerald and Bailey 1984; Romme et al. 1995), but we did not measure such herbivory.

White trunk rot plays an important ecosystem function for cavity-nesting birds and the species that inhabit the cavities after they are abandoned by the excavator (Losin et al. 2006). White trunk rot had an almost equal incidence, near 20%, in healthy and damaged plots, indicating that it was an unlikely contributor to SAD, although the incidence of white trunk rot was greater at low elevations than at high elevations. White trunk rot was more prominent on large than on small trees, consistent with the well-established relationship

between age and decay in aspen (Worrall and Fairweather 2009).

Leafhoppers were unique because their incidence showed a negative relationship with root mortality, plot RCL, Cytospora, and bronze poplar borer. They were also more common in healthy plots than in damaged plots. Leafhoppers usually cause little tree damage except when densities are high and, indeed, most plots with leafhoppers had minimal damage from them.

Although plot RCL is an obvious and useful measure of SAD severity, for several reasons, it may not always correlate strongly with factors related to SAD. First, stands may be on a trajectory toward severe decline but be in the early stages, so RCL is low. Second, stands not affected by SAD can still experience normal mortality and high RCL from unrelated causes. The types and intensity of agent activity in a stand, as characterized by agent communities, may help identify such stands. Classification of plots into agent communities showed several different community types. In the first classification performed, 71 of 81 damaged plots fell into a single group (group 6), indicating a strong agent community type for SAD plots. Secondary agents, such as the three that were consistently dominant in our analysis — bronze poplar borer, Cytospora canker, and bark beetles — showed the greatest incidence in the group 6 classifications. Armillaria and sooty-bark canker also appeared in group 6, but at lower levels. Twenty-two of the 93 plots in group 6 were “healthy” plots, meaning that they had $\leq 25\%$ RCL. These putatively healthy plots may in fact be in the early stages of decline. The classification of group 6 into four different subgroups showed secondary agents in each group but different incidences of those agents. Because agent frequencies increased with levels of crown loss (groups 6a–6d), the subgroups appear to distinguish the stage of decline that the damaged plots are in.

That leaves groups 1–5 of the classification as those characterized less by their association with SAD. Bronze poplar borer, Cytospora canker, and bark beetles show much lower levels in groups 1–5 than in group 6. The remaining dominant agents, including poplar borer and white trunk rot, have their greatest incidences outside of group 6. The agent communities separating groups 1–5 in aspen stands are difficult to assess because this was not a random selection of healthy stands. Classification based on agents may be useful in identifying stands with low RCL in the early stages of SAD but less so in identifying stands with damage due primarily to causes other than SAD because secondary agents play a broad role in attacking trees weakened from multiple causes.

In contrast with recent aspen declines in Alberta and Arizona (Brandt et al. 2003; Fairweather et al. 2008), we found no evidence from aerial survey records of tent caterpillar or any other defoliating insect at substantial levels in our study area in the 10 years prior to the onset of SAD or during the decline. Although intense or repeated defoliations can be inciting factors in declines (Manion and LaChance 1992), severe moisture stress was apparently sufficient to incite the decline of these stands without the additional stress of major insect defoliation.

Our investigation was intended to assess any damage agent in aspen, but there could be cryptic agents present that were not identified. For example, Hibben and Bozarth (1979) found tobacco necrosis virus in declining aspen stands in

Utah, although there was no evidence linking the virus to the decline. Insect-vectored nematodes such as *Bursaphelenchus tryphloei* (Tomalak and Filipiak 2011) could also play a role. We did not look for these agents, but they could be considered in future studies.

What could account for a change in behavior and increased damage by *T. populi* as well as the increased activity of the other agents associated with SAD? Under normal circumstances, these secondary agents are “opportunists”, able to invade and perhaps kill hosts whose defenses are compromised by stressful conditions or reduced storage reserves. As insect or pathogen populations build on such hosts, their numbers or, in the case of pathogens, inoculum potential may increase so that they can attack and overcome more vigorous hosts. In our study, both aspen bark beetles and bronze poplar borer could invade trees with healthier crowns in damaged plots than in healthy plots, suggesting a density-dependent increase in aggressive behavior. Such behavior is well known for many bark beetles (Stock et al. 1992), and increased resource availability can lead to greater inoculum potential and increased frequency of many diseases, including Armillaria root disease (Garrett 1956). Similarly, helminths, myxozoans, bacteria, and fungi can transition from opportunistic to virulent pathogens of fish under elevated resource availability (Wedekind et al. 2010). Thus, two effects are likely at play: environmental stress leading to increased host susceptibility followed by a density-dependent increase in invasive ability. In addition, high temperatures could directly affect agents to increase their success.

Moisture stress often leads to increased tree mortality due to insect and disease activity (Allen et al. 2010). SAD, in particular, was incited by a warm drought of the sort anticipated to become more common under recent climate change projections (Rehfeldt et al. 2009; Worrall et al. 2010). This drought increased the success of some secondary agents of aspen, and this will likely recur in the future if a warmer, dry climate prevails.

Conclusions

As discussed by Manion and LaChance (1992), sometimes a syndrome is loosely termed a decline until a single cause is found. In this instance, however, the dominance of secondary agents, the lack of a widespread primary agent, and the mounting evidence implicating the drought earlier in the decade show this to be a true decline. There is no evidence that the mortality was caused by a primary agent; rather, the outbreak of secondary agents increased the rate of mortality in aspen stands that were already affected by predisposing and inciting factors. As populations of these agents build, some are more able to attack vigorous trees, potentially amplifying the impact of SAD beyond the trees predisposed to severe drought stress and killing trees that may have otherwise recovered. Our results suggest several mechanisms by which environmental stress can increase aggression of insects and diseases.

The prominence of bronze poplar borer, Cytospora canker, and aspen bark beetles in all of our analyses suggests that these insects and pathogen benefitted most from the decline in terms of population increase. Each of these agents damages the phloem and vascular cambium of aspen, which can cause rapid dieback and mortality. This may partly explain

why these agents in particular are linked with crown loss. The future population levels of these agents depend on future climate and (or) other primary pathogen outbreaks.

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